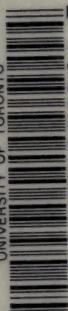


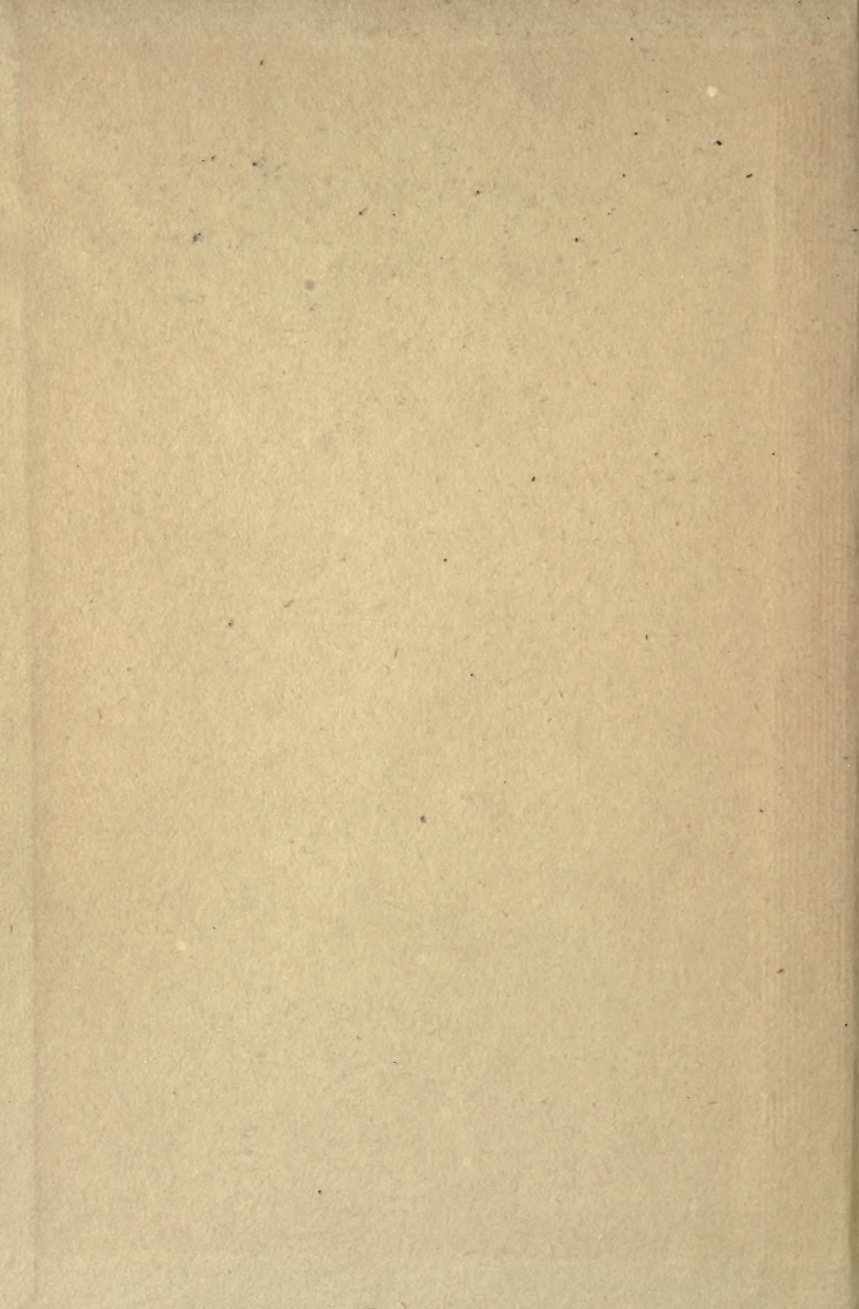
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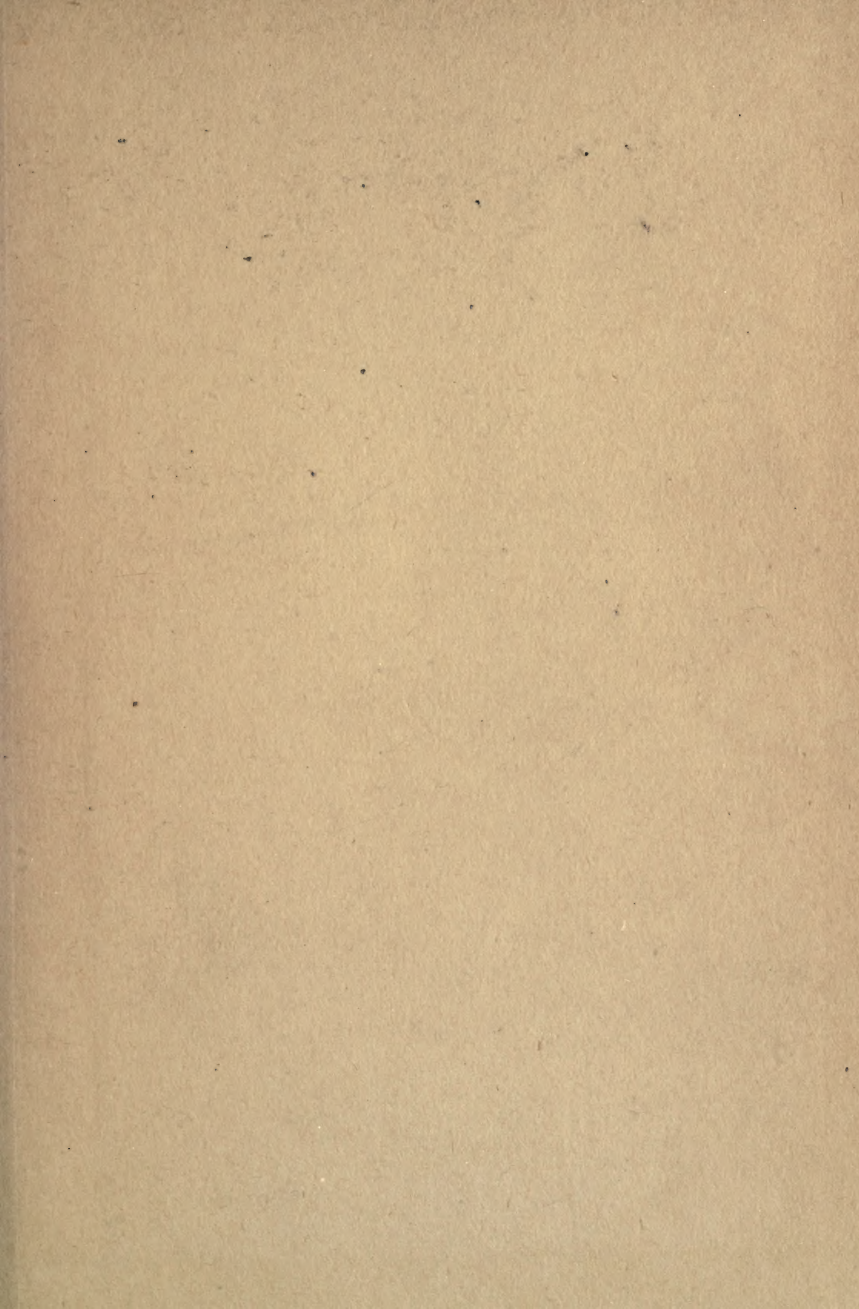
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ANALYSIS OF MILK

AND

MILK PRODUCTS



BY

HENRY LEFFMANN, M. D.

PROFESSOR OF CHEMISTRY IN THE WOMAN'S MEDICAL COLLEGE OF
PENNSYLVANIA AND IN THE WAGNER FREE INSTITUTE OF
SCIENCE OF PHILADELPHIA; PATHOLOGICAL CHEMIST
TO JEFFERSON MEDICAL COLLEGE HOSPITAL

FOURTH EDITION, REVISED AND ENLARGED
WITH ILLUSTRATIONS

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PREPARED AND ISSUED UNDER THE JOINT AUTHOR-
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PREFACE

This book is intended as a guide to the analysis of milk and milk products in the routine of the commercial and food-inspection laboratory. Only processes of practical value have been given, and nothing has been said as to the food value of milk and its products, nor concerning the effects of the several adulterants that may be detected.

A notable portion of the text has been taken from Volume 8 of the Fourth Edition of Allen's *Commercial Organic Analysis*. I am indebted to the courtesy of Messrs. P. Blakiston's Son & Co. for permission to use this matter.

An interesting point is to be noted in the comparison of this edition with the first, issued about a score of years ago in association with Dr. William Beam. In that, a considerable part of the material was derived from the publications of foreign workers; in the present, American investigations form the basis of many of the important processes.

"Westward the star of empire takes its way."

H. L.

PHILADELPHIA.

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MILK

ANALYTIC DATA

Milk, the nutritive secretion of nursing mammals, contains water, fat, proteins, sugar, and mineral matter. Cow's milk is meant in all cases, unless otherwise stated. Milk as taken from the animal is generally termed "whole milk."

Fat.—This occurs in globules varying from 0.0015 mm. to 0.005 mm. in diameter, in a condition which prevents spontaneous coalescence. It is peculiar among animal fats in containing a notable proportion of acid radicles with a small number of carbon atoms.

Proteins.—The nature of the proteins of milk has been much discussed, but it is now generally conceded that there are at least three forms, casein, albumin, and globulin, the casein being present in by far the greatest amount, and the globulin as traces only.

CASEIN.—Casein is probably in part in combination with phosphates. It is precipi-

tated by many substances, among which are acids, rennet, and magnesium sulfate, but not by heat. Acids precipitate it by breaking up the combination with phosphates. The action of rennet is complex and probably partly hydrolytic, splitting the casein into several proteins, some of which are precipitated in the curd. Films of protein matter occur abundantly in milk, for which reason it is distinctly opaque, even when nearly all the fat has been removed by centrifugal action.

The *albumin* of milk appears to be a distinct form, and is called lactalbumin. It is not precipitated by dilute acids, but is coagulated by heating to 70° — 75° . The proportion in cow's milk is usually from 0.35 to 0.50%, but colostrum may contain much larger proportions.

Globulin is present only in minute amounts in normal milk, but colostrum may contain as much as 8%. It is coagulated on heating.

Lactose.—This is a sugar peculiar to milk.

Citric acid is a normal constituent of the milk of various animals. In human milk, the quantity is about 0.5 gram to 1000 c.c.; in cow's milk, from 1 to 1.5 grams. It is not dependent on the citric acid present in the food.

Enzymes.—Several enzymes occur in milk but they are chiefly known by effects and not as isolated substances. Some are proteolytic, others

are oxydases, that is, decompose hydrogen peroxid and carry oxygen over to other substances.

Lecithin is also a usual ingredient of milk. Nerking and Haensel found a range in cows' milk from 0.03 to 0.11%.

Mineral Matter.—The ash of milk contains calcium, magnesium, iron, potassium, and sodium as chlorids, carbonates, sulfates, and phosphates. It does not exactly represent the salts present in milk.

Richmond has determined the ratio of the ash to the solids not fat in 135 samples of milk. This was found to range from 7.8 to 9.4%, but more usually from 7.8 to 8.5 (average 8.2) %. Many ashes were alkaline to turmeric, litmus, and phenolphthalein, the maximum alkalinity being 0.025% calculated as sodium carbonate.

Human milk is notable for a low protein content hence the curd is less bulky and more friable than that from cows' milk. The milk of all animals is subject to modification by breed, climate, season, feed, housing, exercise, time of lactation, and in human beings (and possibly in some other animals) by psychic influences.

As regards the proportion of proteins and lactose, milks of the mare and ass agree closely with human milk.

Normal milk is an opaque white or yellowish-white fluid, with an odor recalling that of the

animal, and a faint sweet taste. The opacity is due largely but not entirely to the fat globules. The reaction of freshly drawn milk to litmus is usually alkaline, but is sometimes amphoteric; that is, it turns the red paper blue and the blue paper red. The sp. gr. varies between 1.027 and 1.035. It usually undergoes a gradual augmentation (sometimes termed Recknagel's phenomenon) for a considerable time after the sample has been drawn. The increase may amount to two units (water being 1000). The sp. gr. becomes stationary in about five hours if the milk is maintained at a temperature below 15°, but at a higher temperature it may require twenty-four hours to acquire constancy. The change is not entirely dependent on the escape of gases.

Unless collected with special care and under conditions of extreme cleanliness, milk always contains many bacteria, animal matter of an offensive character, such as epithelium, blood and pus cells, particles of feces, and soil.

At ordinary temperature milk soon undergoes decomposition, by which the milk sugar is converted principally into lactic acid, and the proteins partly decomposed and partly coagulated. The liquid becomes sour and the fat is inclosed in the coagulated casein. In the initial stages of decomposition the proteins frequently

undergo transformations into substances which are the cause of the violent poisonous effects occasionally produced by ice-cream and other articles of food into the preparation of which milk enters.

Boiling produces coagulation of the albumin, some caramelization of the sugar, and develops a greater facility of coalescence on the part of the fat globules. Enzymes are rendered inert and most microbes are killed.

When milk is allowed to stand, some of the fat rises gradually and forms a rich layer, constituting cream. The proportion of cream depends on several conditions. The amount formed in a given time cannot be taken as a measure of the richness of the milk. Water added to milk causes a more rapid separation of the cream. Centrifugal action separates nearly all of the fat. The following figures, given by D'Hout as averages, show this effect:

	WHOLE MILK	SEPARATED MILK	CREAM
Specific gravity.....	1032	1034	1015
Total solids.....	14.10	9.6	26.98
Sugar.....	4.70	5.05	3.32
Casein.....	3.50	3.62	2.02
Ash.....	0.79	0.78	0.58
Fat.....	5.05	0.20	21.95

Buttermilk is the residue after removal of the butter by churning. Vieth gives the following analyses:

TOTAL SOLIDS	FAT	SOLIDS NOT FAT	Ash
9.03	0.63	8.40	0.70
8.02	0.65	7.37	1.29
10.70	0.54	10.16	0.82

Whey or *Milk-serum* is the liquid freed from curd after precipitation by rennet or acids. In most cases it contains a notable amount of proteins, as shown in the following analyses by Cochran:

MILK		WHEY	
Total solids	Solids not fat	Total solids	Proteins removed
9.27	9.13	6.62	2.51
9.27	9.13	6.1	3.03
14.05	8.35	6.62	2.33
7.71	7.61	5.98	1.63
8.91	8.71	6.50	2.21

The whey of any given milk has practically the same composition, whether taken from the original milk, skimmed milk, or cream.

Average Proportion of Solids in Milk.—The most extensive data on this point are those obtained by Vieth. The total number of samples was 120,540. The averages of the entire series are as follows:

Fat.....	4.1%
Non-fatty solids.....	8.8%
Total solids.....	12.9%

Lythgoe gives a table of averages of composition of 51 samples of genuine milk, each set of

averages being deduced by analysis of 10 samples. The following data are selected from this table. For explanation of the figures in the last column see page 42.

TOTAL SOLIDS	FAT	PRO- TEINS	LAC- TOSE	ASH	SOLIDS NOT FAT	REFRACTION OF COPPER SERUM 20°
15.70	6.01	4.13	4.79	0.77	9.69	38.1
15.00	5.62	3.75	4.87	0.76	9.38	38.3
14.50	5.30	3.61	4.82	0.77	9.20	38.3
14.00	4.78	3.51	4.98	0.73	9.22	38.5
13.50	4.61	3.37	4.77	0.75	8.89	38.1
13.00	4.24	3.17	4.86	0.73	8.76	37.9
12.50	3.99	2.84	4.94	0.73	8.51	38.0
12.00	3.45	2.88	4.96	0.74	8.55	37.7
11.50	3.33	2.67	4.80	0.70	8.17	37.3
11.00	3.02	2.64	4.63	0.71	7.98	37.0
10.70	2.90	2.60	4.49	0.71	7.80	36.4

From these figures Lythgoe derives the rule that differences in proportion of solids not fat in unadulterated milks are principally due to differences in the amount of proteins. Lactose and ash are fairly constant. On these facts depend recently introduced methods of detecting watering milk, as will be pointed out later.

Colostrum.—This is the secretion in the early stages of lactation, and differs from ordinary milk. It contains characteristic structures, known as colostrum corpuscles, and usually contains much less fat than fully developed milk, but a larger proportion of proteins. Colostrum coagulates on boiling. Lactose is in small amount.

ANALYTIC PROCESSES

Specific Gravity.—The sp. gr. of milk rises gradually for some time after it has been drawn, and the determination is to be made only after this action has ceased. This will require about five hours after the milk is drawn, if it has been kept 15°, but at a higher temperature it will be necessary to allow at least twelve hours. For all other determinations the milk must be analyzed as soon as possible. The following figures, published by Bevan, show that a considerable loss in total solids may occur in twenty-four hours:

	TOTAL SOLIDS	LOSS
Evaporated immediately ...	11.73	
Evaporated after 24 hours,	10.79	0.94
Evaporated after 48 hours,	10.38	1.35
Evaporated after 120 hours,	9.42	2.31

The decomposition is very irregular, and it is not possible to determine, by estimation of the lactic acid or other products, the original composition of the milk.

Air-bubbles are held rather tenaciously by milk, and care must be taken in mixing, preparatory to taking the sp. gr., to avoid as far as possible

the inclosure of the air, and to allow sufficient time for the escape of any bubbles that may be present. Sp. gr. is understood to be taken at 15.5° ; samples should be brought near to this. If at a few degrees above or below, it will suffice to make the determination at once and obtain the correct figure by reference to the annexed table. The sp. gr. of normal milk ranges between 1.027 and 1.035. The figure alone does not indicate the character of the sample, but taken in conjunction with the figure for fat or for total solids, it is of value as a check on the results furnished by other determinations.

The simplest method of determining sp. gr. is by the *lactometer*, a delicate and accurately graduated hydrometer. The instrument must be immersed carefully so as not to wet the stem above the point at which it will rest. Its accuracy should be verified by immersion in distilled water at 15.5° and milks of known sp. gr.

More accurate determinations may be made with a balance. A special form, the Westphal balance, is adapted to the determination of sp. gr. only, the weights being so arranged that a simple enumeration of them gives the gravity directly. The cheap forms of this instrument are not satisfactory, but some made by German houses are excellent. The ordinary

Find the temperature of the milk in one of the horizontal lines and the sp. gr. in the first vertical column. In the same line with this and the temperature the correct figure is given.

°F.	50	51	52	53	54	55	56	57	58	59	60	61	62
Sp.Gr.													
21	20.2	20.3	20.3	20.4	20.5	20.6	20.7	20.8	20.9	20.9	21.0	21.1	21.2
22	21.2	21.3	21.3	21.4	21.5	21.6	21.7	21.8	21.9	21.9	22.0	22.1	22.2
23	22.2	22.3	22.3	22.4	22.5	22.6	22.7	22.8	22.8	22.9	23.0	23.1	23.2
24	23.2	23.3	23.3	23.4	23.5	23.6	23.6	23.7	23.8	23.9	24.0	24.1	24.2
25	24.1	24.2	24.3	24.4	24.5	24.6	24.6	24.7	24.8	24.9	25.0	25.1	25.2
26	25.1	25.2	25.2	25.3	25.4	25.5	25.6	25.7	25.8	25.9	26.0	26.1	26.2
27	26.1	26.2	26.2	26.3	26.4	26.5	26.6	26.7	26.8	26.9	27.0	27.1	27.3
28	27.0	27.1	27.2	27.3	27.4	27.5	27.6	27.7	27.8	27.9	28.0	28.1	28.3
29	28.0	28.1	28.2	28.3	28.4	28.5	28.6	28.7	28.8	28.9	29.0	29.1	29.3
30	29.0	29.1	29.1	29.2	29.3	29.4	29.6	29.7	29.8	29.9	30.0	30.1	30.3
31	29.9	30.0	30.1	30.2	30.3	30.4	30.5	30.6	30.8	30.9	31.0	31.2	31.2
32	30.9	31.0	31.1	31.2	31.3	31.4	31.5	31.6	31.7	31.9	32.0	32.2	32.3
33	31.8	31.9	32.0	32.1	32.3	32.4	32.5	32.6	32.7	32.9	33.0	33.2	33.3
34	32.7	32.9	33.0	33.1	33.2	33.3	33.5	33.6	33.7	33.9	34.0	34.2	34.3
35	33.6	33.8	33.9	34.0	34.2	34.3	34.5	34.6	34.7	34.9	35.0	35.2	35.3
°C.	10.0	10.5	11.1	11.6	12.2	12.7	13.3	13.8	14.4	15.0	15.5	16.1	16.6

analytic balance may also be used. A plummet consisting of a thick glass rod (or short sealed tube, weighted with mercury) of a bulk of about 10 c.c. is suspended from the hook of the balance by means of fine platinum wire and the weight ascertained. It is then submerged in distilled water and the weight also noted. The water is contained in a narrow upright cylinder resting

Find the temperature of the milk in one of the horizontal lines and the sp. gr. in the first vertical column. In the same line with this and the temperature the correct figure is given.

63	64	65	66	67	68	69	70	71	72	73	74	75
21.3	21.4	21.5	21.6	21.7	21.8	22.0	22.1	22.2	22.3	22.4	22.5	22.6
22.3	22.4	22.5	22.6	22.7	22.8	23.0	23.1	23.2	23.3	23.4	23.5	23.7
23.3	23.4	23.5	23.6	23.7	23.8	24.0	24.1	24.2	24.3	24.4	24.6	24.7
24.3	24.4	24.5	24.6	24.7	24.9	25.0	25.1	25.2	25.3	25.5	25.6	25.7
25.3	25.4	25.5	25.6	25.7	25.9	26.0	26.1	26.2	26.4	26.5	26.6	26.8
26.3	26.5	26.6	26.7	26.8	27.0	27.1	27.2	27.3	27.4	27.5	27.7	27.8
27.4	27.5	27.6	27.7	27.8	28.0	28.1	28.2	28.3	28.4	28.6	28.7	28.9
28.4	28.5	28.6	28.7	28.8	29.0	29.1	29.2	29.4	29.5	29.7	29.8	29.9
29.4	29.5	29.6	29.8	29.9	30.1	30.2	30.3	30.4	30.5	30.7	30.9	31.0
30.4	30.5	30.7	30.8	30.9	31.1	31.2	31.3	31.5	31.6	31.8	31.9	32.1
31.4	31.5	31.7	31.8	32.0	32.2	32.2	32.4	32.5	32.6	32.8	32.0	33.1
32.5	32.6	32.7	32.9	33.0	33.2	33.3	33.4	33.6	33.7	33.9	23.0	34.2
33.5	33.6	33.8	33.9	34.0	34.2	34.3	34.5	34.6	34.7	34.9	35.1	35.2
34.5	34.6	34.8	34.9	35.0	35.2	35.3	35.5	35.6	35.8	36.0	36.1	36.3
35.5	35.6	35.8	35.9	36.1	36.2	36.4	36.5	36.7	36.8	37.0	37.2	37.3
17.2	17.7	18.3	18.8	19.4	20.0	20.5	21.1	21.6	22.2	22.7	23.3	23.8

on a bench or support above the scale pan. The loss of weight of the plummet is, of course, the weight of the bulk of water that it displaces. The sp. gr. of any sample can be determined by weighing the plummet immersed in the sample and dividing the loss in weight by the loss in water. The quotient is the sp. gr.

The ordinary pyknometer is not convenient

on account of the liability of the upper layer of the liquid to be richer in fat than the lower; the overflow, therefore, does not represent the mixture.

Total Solids.—This determination may often be made with sufficient accuracy for practical purposes by evaporating a measured volume (*e. g.*, 3 or 5 c.c.) in a shallow nickel dish from 5 to 8 cm. in diameter. Nickel crucible-covers are suitable. The thin glass (Petri) dishes used for microbe culture are convenient. When greater accuracy is required, and especially when the ash is to be determined, platinum dishes are the most satisfactory, but owing to the present price of this metal, quartz dishes are now much used. Either the translucent or transparent quartz is suitable, the former being less expensive.

Good results may be secured as follows: A flat dish, 3.5 cm. in diameter, with sides 0.5 cm. high, is provided with a thin flat watch-glass cover that fits rather closely. The total weight of the cover and dish is noted. 2 or 3 c.c. of the sample are run into the dish from the pipet, the watch-glass placed on, and the weight taken as rapidly as possible. The glass prevents appreciable loss from evaporation during an ordinary weighing. The cover is removed, the dish heated on the water-bath or in the water-oven, and weighed from time to time (with cover on it) until the

weight is sensibly constant. The percentage of residue can be easily calculated. About three hours may be required to secure constant weight.

When high accuracy is not essential, it will suffice to measure the milk. Vieth advised a pipet graduated to deliver 5 grams, and found that, working with whole and skimmed milk, under the ordinary variations of temperature, the error will not exceed 0.1 on the total solids and less on the fat. The pipet should have a rather wide opening so that no cream will be retained.

The Massachusetts State Board of Health has for many years used the routine method of evaporating 5 grams for two hours in a flat platinum basin over boiling water.

The A. O. A. C. method is: Heat at 100° to constant weight, about 3 grams in a tared platinum, aluminum or tin dish of 5 cm. diameter, with or without the addition of 15 to 30 grams of sand. Cool and weigh.

Ash.—The residue from the determination of total solids is heated cautiously over the Bunsen burner, until a white ash is left. The result obtained in this manner is apt to be slightly low from loss of sodium chlorid. This may be avoided by heating the residue sufficiently to char it, extracting the soluble matter with a few c.c. of water, and filtering (using paper extracted

with hydrofluoric acid). The filter is added to the residue, the whole ashed, the filtrate then added, and the liquid evaporated carefully to dryness. The ash of normal milk is about 0.7 % and faintly alkaline. A marked degree of alkalinity and effervescence with hydrochloric acid will suggest the addition of a carbonate.

The method of the A. O. A. C. is as follows: In a weighed dish put 20 c.c. of milk from a weighing bottle; add 6 c.c. of nitric acid, evaporate to dryness, and burn at a low red heat till the ash is free from carbon.

Fat.—Many methods for fat determination have been devised. The following will suffice for all practical work:

Adams' Method.—This consists essentially in spreading the milk over absorbent paper, drying, and extracting the fat in an extraction apparatus; the milk is distributed in an extremely thin layer, and by a selective action of the paper the larger portion of the fat is left on the surface. A paper, manufactured especially for this purpose by Schleicher & Schuell, is obtainable in strips of suitable size. Each of these yields to ether only from 0.001 to 0.002 gram of extract.

Coils made of thick filter-paper, cut into strips 6 by 62 cm., are thoroughly extracted with ether and alcohol, or the weight of the extract corrected by a constant obtained for the

paper. From a weighing bottle about 5 grams of the milk are transferred to the coil by means of a pipet, care being taken to keep dry the end of the coil held in the fingers. The coil is placed, dry end down, on a piece of glass and dried for one hour, preferably in an atmosphere of hydrogen; it is then transferred to an extraction apparatus and extracted with absolute ether, petroleum spirit of boiling-point about 45° or, better, carbon tetrachlorid. The extracted fat is dried and weighed.

The above procedure is very satisfactory, but the drying in hydrogen may usually be omitted. After the coil has received at least twenty washings, the flask is detached, the ether removed by distillation, and the fat dried by heating in an air-oven at about 105° , and occasionally blowing air through the flask. After cooling, the flask is wiped with a piece of silk, allowed to stand ten minutes, and weighed.

Richmond states that to perform a rigidly accurate determination attention to the following points is necessary: The ether must be anhydrous (drying over calcium chlorid and distilling is sufficient). Schleicher & Schuell's fat-free papers should be used, and one should be extracted without any milk on it, as a tare for the others. Four or five hours' extraction is necessary, and

the coils should be well dried before extraction is begun.

Thimble-shaped cases made of fat-free paper are now obtainable and are convenient for holding the absorbent material on which the milk is spread. The fine texture prevents undissolved matter escaping. A case may be used repeatedly. Sour milk may be thinned with ammonium hydroxid before taking the portion for analysis.

Babcock Asbestos Method.—This is recommended by the A. O. A. C.: Provide a hollow cylinder of perforated sheet metal 60 mm. long and 20 mm. in diameter, closed 5 mm. from one end by a disk of the same material. The perforations should be about 0.7 mm. in diameter and 0.7 mm. apart. Fill the cylinder loosely with from 1.5 to 2.5 grams of freshly ignited woolly asbestos free from fine or brittle material. Cool in a desiccator and weigh. Introduce a weighed quantity of milk (about 4 grams) and dry at 100°. The cylinder is placed in the extraction tube and extracted with ether in the usual way. The ether is evaporated and the fat weighed. The extracted cylinder may be dried at 100° and the fat checked by the loss in weight. A higher degree of accuracy is secured by performing the drying operation in hydrogen.

For thorough extraction, especially with difficulty soluble materials and volatile solvents, the

continuous extraction apparatus devised by Szombathy, but commonly called the Soxhlet tube, is most suitable.

The material may be placed in a fat-free paper thimble and covered with a plug of cotton to prevent loss of fine particles. In place of the cotton plug, a porcelain or platinum Gooch crucible may be used, as shown in the cut. The top of the thimble should be a short distance below, and the top of the crucible a short distance above, the bend of the siphon. The thimble should be supported by a section of glass tubing, 1 to 2 cm. long, with rounded edges; the edge on which the thimble rests should be a little uneven to prevent a close joint, which would hinder the siphoning of some of the liquid.

Alundum cylinders will probably be useful.

Loss of solvent by leakage often occurs. It may be diminished somewhat by soaking the corks in rather strong hot gelatin solution, draining them quickly and then exposing them for some hours to formaldehyde vapor.

The solvents most generally employed are ether and petroleum spirit, but carbon tetrachlorid is well adapted for extraction purposes as it has high solvent power for fats and is not easily inflammable.

When extraction is completed, the carton and

materials may be removed from the tube, and, replacing the parts of the apparatus, much of the solvent may be redistilled into the extractor, thus recovering the liquid. Care must be taken not to distil the contents of the flask closely or heat strongly, lest some of the more volatile of the dissolved matters pass into the distillate.

Roese-Gottlieb Method.—This is now being used for milk-products as well as for milk. For detailed description, see page 72.

Centrifugal Methods.—Although almost all the fat of milk may be separated by the centrifuge, the emulsion is not destroyed and the volume of cream is merely suggestive as to the fat-content of the milk. To obtain a clear fatty layer in condition for close measurement it is necessary to use chemicals. The methods at present most employed depend essentially on one devised by Gustaf DeLaval, who took out a patent in Sweden for the use of a mixture of twenty volumes of strong acetic acid and one volume of strong sulfuric acid. This mixture coagulates and then dissolves the proteins, destroys the emulsion, but does not otherwise affect the fat and does not act on the lactose. By brief whirling in a centrifuge the fat collects in a clear sharply defined layer. DeLaval took out patents in several countries subsequent to the above date.

Leffmann and Beam devised a method in which

a small amount of amyl alcohol with an equal volume of hydrochloric acid was added to the milk, and the proteins thus coagulated dissolved by strong sulfuric acid. About the same time Babcock devised a process in which sulfuric acid was used alone. Subsequently Gerber published a process in which the essential feature of the Leffmann-Beam method, namely, the use of amyl alcohol, was advised.

The test-bottles have a capacity of about 30 c.c. and are provided with a graduated neck, each division of which represents 0.1% by weight of butter fat.

15 c.c. of the milk are measured into the bottle, 3 c.c. of a mixture of equal parts of amyl alcohol and strong hydrochloric acid added, mixed, the bottle filled nearly to the neck with concentrated sulfuric acid, and the liquids mixed by holding the bottle by the neck and giving it a gyratory motion. The neck is now filled to about the zero point with a mixture of sulfuric acid and water prepared at the time. It is then placed in the centrifugal machine, which is so arranged that when at rest the bottles are in a vertical position. If only one test is to be made, the equilibrium of the machine is maintained by means of a test-bottle, or bottles, filled with a mixture of equal parts of sulfuric acid and water. After rotation for from one to two minutes, the

fat will collect in the neck of the bottle and the percentage may be read off. It is convenient to use a pair of dividers in making the reading. The legs of these are placed at the upper and lower limits respectively of the fat, allowance being made for the meniscus; one leg is then placed at the zero point and the reading made with the other. Experience by analysts in various parts of the world has shown that with properly graduated bottles the results are reliable. As a rule, they do not differ more than 0.1 % from those obtained by the Adams process, and are generally even closer.

For great accuracy, the factor for correcting the reading on each of the bottles should be determined by comparison with the figures obtained by the Adams or other standard process.

Cream is to be diluted to exactly ten times its volume, the sp. gr. taken, and the liquid treated as a milk. Since in the graduation of the test-bottles a sp. gr. of 1.030 is assumed, the reading must be increased in proportion.

A more accurate result may be obtained by weighing in the test-bottle about 2 c.c. of the cream and diluting to about 15 c.c. The reading obtained is to be multiplied by 15.45 and divided by the weight in grams of cream taken.

The mixture of fusel oil and hydrochloric acid seems to become less satisfactory when long

kept. It should be clear and not very dark in color. It is best kept in a bottle provided with a pipet which can be filled to the mark by dipping. Rigid accuracy in the measurement is not needed.

[The Leffmann-Beam method is often erroneously called the "Beimling" method, but Beimling was merely the deviser of a cheap centrifuge. To protect the interest of a manufacturer who had invested in the Beimling machine under the impression that it was a practicable method for fat estimation, it became necessary for Leffmann and Beam to take out a patent (now expired) and assign the same to this investor.]

Calculation Methods.—Several investigators have proposed formulæ by which when any two of the data, sp. gr., fat, and total solids, are known, the third can be calculated. These differ according to the method of analysis employed. That of Hehner and Richmond, as corrected by Richmond, was deduced from results by the Adams method of fat extraction. It is:

$$T = 0.25 G + 1.2 F + 0.14;$$

in which T is the total solids, G the last two figures of the sp. gr. (water being 1000), and F the fat. Patrick has proved that with American milks the constant should be dropped, the formula reading:

$$T = 0.25 G + 1.2 F$$

Babcock's formula has been much used in the United States. It is adapted to calculating the solids not fat. In this formula g is the entire figure for sp. gr. referred to water as 1.

$$S_{nf} = \left(\frac{100g - fg}{100 - 1.0753fg} - 1 \right) \times 2.5 (100 - f)$$

Babcock has also given a much simpler form adapted for total solids. This differs but slightly from Richmond's.

Total Proteins.—3 types of processes are employed for this estimation: Calculation from the total nitrogen; precipitation and direct weighing; calculation from the "aldehyde-figure." Milk contains appreciable amounts of non-protein nitrogen, but the fact is usually disregarded. According to Munk, this may range, in cow's milk, from 0.022 to 0.034%, and from 0.014 to 0.026% in human milk. By these figures, the average protein nitrogen in cow's milk would be 94%, and in human milk 91%, of the total nitrogen.

Kjeldahl-Gunning Method.—(Calculation from total nitrogen).

Reagents:

Potassium sulfate.—A coarsely powdered form free from nitrates and chlorids should be selected.

Sulfuric acid.—This should have a sp. gr. 1.84 and be free from nitrates and ammonium.

Standard acid.— $N/2$ Sulfuric or hydrochloric acid, the strength of which has been accurately determined.

Standard alkali.— $N/10$ Ammonium hydroxid, sodium hydroxid, or barium hydroxid, the strength of which in relation to the standard acid must be accurately determined.

Sodium hydroxid solution.—500 grams should be added to 500 c.c. of water, the mixture allowed to stand until the undissolved matter settles, the clear liquor decanted and kept in a stoppered bottle. It will be an advantage to determine approximately the quantity of this solution required to neutralize 20 c.c. of the strong sulfuric acid.

Indicator.—Cochineal solution is recommended by the A. O. A. C., but methyl-orange and sodium alizarin-monosulfonate are satisfactory. Methyl-orange solution should be very dilute; 1 part in 1000. A drop is sufficient for 100 c.c. of liquid. Phenolphthalein is not well adapted to tritiation of ammonium compounds.

Digestion and distillation flasks.—Jena-glass round-bottomed flasks with a bulb 12.5 cm. long and 9 cm. in diameter, the neck cylindrical, 15 cm. long and 3 cm. in diameter, flared slightly at the mouth.

Process

5 c.c. of the sample are placed in a digestion

flask, 10 grams of powdered potassium sulfate and 15 to 25 c.c. (ordinarily about 20 c.c.) of the strong sulfuric acid are added and the digestion conducted as follows: The flask is placed in an inclined position and heated below the boiling-point of the acid for from five to fifteen minutes, or until frothing has ceased. Excessive frothing may be prevented by the addition of a small piece of paraffin. The heat is raised until the acid boils briskly. A small, short-stemmed funnel may be placed in the mouth of the flask to restrict the circulation of air. No further attention is required until the liquid has become clear and colorless, or not deeper than a pale straw.

When Kjeldahl operations are carried out in limited number, the arrangement used in my laboratory has been found very satisfactory. A double-Y, terra cotta drain-pipe, about 20 cm. internal diameter, is connected by an elbow directly with the chimney-stack. The digestion flasks are supported as shown in the rough sketch, figure 1 (not drawn exactly to scale). Two flasks can be operated at once. The central opening is convenient for other operations producing fumes. Openings not in use are closed by circles of heavy asbestos.

Apparatus for use when many determinations are made are figured in the catalogs of supply-

houses. As corrosive vapors are given off, it must be placed under a hood; but a special form of apparatus is now made which does not require an escape-pipe.

When the liquid has become colorless or very light straw yellow, it is allowed to cool, diluted with 100 c.c. of water if the smaller form of

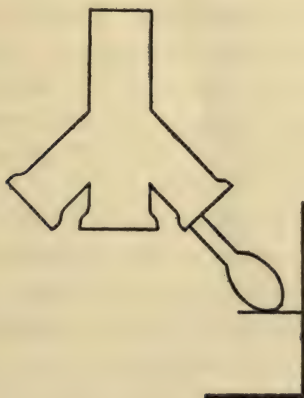


FIG. 1.

flask has been used, the liquid transferred to the distilling flask, and the digestion flask rinsed with two portions of water, 50 c.c. each, which are also transferred to the distilling flask. With the larger form of flask the dilution is made at once by the cautious addition of 200 c.c. of water. Granulated zinc, pumice stone, or 0.5

gram of zinc dust is added. 50 c.c. of the strong sodium hydroxid solution, or sufficient to make the reaction strongly alkaline, should be slowly poured down the side of the flask so as not to mix at once with the acid solution. It is convenient to add to the acid liquid a few drops of phenolphthalein or azolitmin solution, to indicate when the liquid is alkaline, but it must be noted that strong alkaline solutions destroy the former indicator. The flask is shaken so as to mix the alkaline and acid liquids and at once attached to the condensing apparatus. The receiving flask should have been previously charged with a carefully measured volume of the $N/2$ acid (10 c.c. diluted with distilled water to 100 c.c. is a convenient amount). The distillation is conducted until about 150 c.c. have passed over. A small amount of indicator is added, the liquid, titrated with standard alkali, and the amount neutralized by the distilled ammonium hydroxid determined by subtraction. Each c.c. of $N/2$ acid neutralized is equivalent to 0.007 nitrogen. The nitrogen multiplied by 6.38 gives the *total proteins*.

The distillation in this operation requires care, as the amount of ammonium hydroxid is determined by its neutralizing power, hence solution of the alkali of the glass will introduce error. Common glass is not satisfactory. Block

tin is a good material. Moerrs found that Jena-glass tubes resist the action of the ammonium hydroxid. Distillates should be titrated promptly as alkali may be dissolved from the glass.

A satisfactory condensing arrangement for general laboratory use is a copper tank of good size, through which several condensing tubes pass.

Aldehyde Number.—The addition of formaldehyde to milk increases the acidity by an action on the proteins. As commercial formaldehyde is always acid, the acidity must be either determined or neutralized in applying the following method. The application of the reaction to determination of proteins in milk is due to Steinegger. Richmond and Miller investigated the method and suggested the use of strontium hydroxid instead of sodium hydroxid. Richmond gives the following details:

To 10 c.c. of milk at least 1 c.c. of a 0.5% solution of phenolphthalein is added and the liquid neutralized with standard strontium hydroxid solution. To the faintly pink liquid, 2 c.c. or more of 40% formaldehyde solution are added and the titration made to the same tint as the former. The strontium hydroxid required by the formaldehyde solution must be known, and this being deducted from that which was used in the titration and the remainder calculated

to c.c. $N/1$ acid per 1000 c.c. of milk will give the "aldehyd number." Richmond finds that this multiplied by 0.17 gives in most cases a close approximation to the total proteins obtained by the Kjeldahl method.

Calculation Method.—Olson has shown that in normal milks the proteins may be calculated with close approximation by the formula

$$p = t - \frac{t}{1.34}$$

in which p is protein and t total solids.

DETERMINATION OF SPECIAL PROTEINS.—*Casein* and *albumin* may be determined by Sébelein's method: 20 c.c. of the sample are mixed with 40 c.c. of a saturated solution of magnesium sulfate and powdered magnesium sulfate stirred in until no more will dissolve. The precipitate of casein and fat, including the trace of globulin, is allowed to settle, filtered, and washed several times with a saturated solution of magnesium sulfate. The filtrate and washings are saved for the determination of albumin. The filter and contents are transferred to a flask and the nitrogen determined by the method described above. The nitrogen so found, multiplied by 6.38, gives the casein.

The filtrate and washings from the determina-

tion of casein are mixed, the albumin precipitated by *Almén's tannin reagent*, filtered, and the nitrogen in the precipitate determined as above. The same factor is used.

Almén's reagent is prepared by dissolving 4 grams of tannin in 190 c.c. of 50% alcohol and adding 8 c.c. of acetic acid of 25%.

In a mixture of milk and whey (prepared with rennet) in about equal parts, Richmond and Boseley found about 0.3% of albumoses not precipitated by the copper sulfate nor by magnesium sulfate, but precipitable, along with the albumin, by a solution of tannin. The separation may be effected by diluting the filtrate from the magnesium sulfate precipitation, acidifying slightly with acetic acid, and boiling, when the albumin will be coagulated and precipitated. The albumoses may be separated by filtering the solution and precipitating with tannin solution. The precipitated proteins are best estimated by determining the nitrogen in the moist precipitate. The separation of the proteins may be effected, though less accurately, but the use of acetic acid, as recommended by Hoppe-Seyler and Ritthausen.

Leffmann and Beam have modified the process to avoid the delay and trouble of washing the precipitate, as follows: 10 c.c. of the milk are mixed with saturated magnesium sulfate solu-

tion and the powdered salt added to saturation. The mixture is washed into a graduated measure with a small amount of the saturated solution, made up to 100 c.c. with the same solution, mixed, and allowed to stand until the separation takes place. As much as possible of the clear portion is drawn off with a pipet and passed through a dry filter. An aliquot portion of the filtrate is taken, the albumin precipitated by a solution of tannin, and the nitrogen in the precipitate ascertained as above.

The following are A. O. A. C. methods:

1. *Provisional Method for the Determination of Casein in Cows' Milk.*—The determination should be made when the milk is fresh. When it is not practicable to make the determination within twenty-four hours, add one part of formaldehyd to 2500 parts of milk and keep in a cool place. 10 grams of the sample are diluted with about 90 c.c. of water at between 40° and 42°, 1.5 c.c. of a solution containing 10% of acetic acid by weight added, allowed to stand for five minutes, washed three times by decantation, pouring the washings through a filter, and the precipitate transferred completely to the filter. If the filtrate is not clear at first, it will generally become so in two or three filtrations, after which the washing can be completed. The nitrogen in the washed precipitate and filter is determined by the

Kjeldahl-Gunning method. The nitrogen, multiplied by 6.38, gives the casein.

In working with milk which has been kept with preservatives, the acetic acid should be added in small portions, a few drops at a time with stirring, and the addition continued until the liquid above the precipitate becomes clear or nearly so.

2. *Provisional Method for the Determination of Albumin in Milk.*—The filtrate obtained in the above operation is neutralized with sodium hydroxid, 0.3 c.c. of the 10% solution of acetic acid added, and the mixture heated for fifteen minutes. The precipitate is collected on a filter, washed, and the nitrogen determined.

Van Slyke has pointed out that the casein can be approximately ascertained by multiplying the figure for total proteins by 0.8.

Modified Proteins, Amino-derivatives and Ammonium Compounds.—The following procedures are given by Van Slyke. The filtrate from the albumin precipitate is heated to 70°, 1 c.c. of 5% sulfuric acid added, then solid zinc sulfate to saturation. The mixture is allowed to stand at 70° until the caseoses settle. The liquid is cooled, filtered, the precipitate washed with saturated solution of zinc sulfate slightly acidified with sulfuric acid and the nitrogen ascertained by the Kjeldahl method.

For amino-derivatives and ammonium compounds, 50 c.c. of the milk are mixed in a flask marked at 250 c.c. with 1 gram of sodium chlorid. A 12 % solution of tannin is added, drop by drop, until no further precipitation occurs. The mixture is diluted to the mark, shaken and filtered through a dry filter. For amino-derivatives, 50 c.c. of the filtrate are treated for nitrogen in the usual way. For ammonium compounds, 100 c.c. of the filtrate are mixed with magnesium oxid and about 50 c.c. distilled, the distillate being received in a known volume of standard acid. Large excess of magnesium oxid must be avoided.

Lactose.—For this determination, A. O. A. C. employs Soxhlet's method with the following reagents:

Copper sulfate solution.—34.639 grams of pure crystallized copper sulfate are dissolved in water and made up to 500 c.c.

Alkaline tartrate solution.—173 grams of pure sodium potassium tartrate and 50 grams of good sodium hydroxid are dissolved in water and the solution made up to 500 c.c.

Sodium hydroxid $N/2$.

25 c.c. of the sample in a 500 c.c. flask are diluted with 400 c.c. of water and 10 c.c. of the copper sulfate solution and 8.8 c.c. $N/2$ sodium hydroxid solution added. The mixture should

still have an acid reaction and contain copper in solution. If this is not the case, the experiment must be repeated, using a little less of the alkali. The flask is filled to the mark with water, shaken, and the liquid passed through a dry filter. 50 c.c. of Fehling's solution, obtained by mixing equal parts of the above copper sulfate and alkaline tartrate solutions, are heated to brisk boiling in a 300 c.c. beaker, 100 c.c. of the filtrate obtained as above added, and boiling continued for six minutes; the liquid then promptly filtered, and treated according to methods given below. The amount of lactose is calculated by the table on page 34 from the copper obtained by table. The figures for weights of copper between any two data given in the table may be calculated with sufficient accuracy for practical purposes by allowing 0.0008 gram of lactose for each 0.001 gram of copper.

The precipitated cuprous oxid is usually converted into free copper and weighed as such. Two methods may be employed for reduction: by hydrogen or by electrolysis.

Reduction by Hydrogen.—The cuprous oxid is collected on an asbestos filter. This is arranged most conveniently in a special filtering tube, which is shown in figure 2. The wider part is about 8 cm. and 1.5 cm. in diameter, the narrower portion about 5 cm. long and 0.5 cm. in caliber.

A perforated platinum disk is sealed in just above the point of narrowing. The asbestos is placed on this disk, washed free from loose fibers, dried well, and the tube weighed. The filtering tube is attached to an exhaustion apparatus by passing narrower portion through the cork, and a

COPPER	LACTOSE	COPPER	LACTOSE	COPPER	LACTOSE
0.100	0.072	0.205	0.151	0.305	0.228
0.105	0.075	0.210	0.154	0.310	0.232
0.110	0.079	0.215	0.158	0.315	0.236
0.115	0.083	0.220	0.162	0.320	0.240
0.120	0.086	0.225	0.165	0.325	0.244
0.125	0.090	0.230	0.169	0.330	0.248
0.130	0.094	0.235	0.173	0.335	0.252
0.135	0.097	0.240	0.177	0.340	0.256
0.140	0.101	0.245	0.181	0.345	0.260
0.145	0.105	0.250	0.185	0.350	0.264
0.150	0.109	0.255	0.189	0.355	0.268
0.155	0.112	0.260	0.192	0.360	0.272
0.160	0.116	0.265	0.196	0.365	0.276
0.165	0.120	0.270	0.200	0.370	0.280
0.170	0.124	0.275	0.204	0.375	0.285
0.175	0.128	0.280	0.208	0.380	0.289
0.180	0.132	0.285	0.212	0.385	0.293
0.185	0.134	0.290	0.216	0.390	0.298
0.190	0.139	0.295	0.221	0.395	0.302
0.195	0.141	0.300	0.224	0.400	0.306
0.200	0.147				

small funnel is fitted tightly in the top of the tube. The object of this funnel is to prevent the precipitate collecting on the upper part of the tube. The lower end of the funnel should project several centimeters below the bottom of the cork through which it passes.

The filtering apparatus must be arranged prior to the precipitation, so that the cuprous oxid may be filtered without delay. The precipitate is transferred as rapidly as possible to the filter, well washed with hot water, alcohol, and ether successively, dried, and the cuprous oxid reduced by gentle heating in a current of hydrogen. When the reduction is complete, the heat is withdrawn, but the flow of hydrogen is continued until the tube is cold. It is then detached and weighed.

Reduction of Copper by Electrolysis.—The filtration is performed in a Gooch crucible with an asbestos-felt film and the beaker in which the precipitation was made is well washed with hot water, the washings being passed through the filter, but it is not necessary to transfer all the precipitate. When the asbestos film is completely washed, it is transferred with the adhering oxid to the beaker; any oxid remaining in the crucible is washed into the beaker by use of 2 c.c. nitric acid (sp. gr. 1.42), added with a pipet. The crucible is rinsed with a spray of

water, the rinsings being collected in the beaker. The liquid is heated until all the copper is in solution, filtered, the filter washed until the filtrate amounts to at least 100 c.c., and electrolyzed.

Electrolytic apparatus has been constructed in a great variety of forms. When the operation is carried out frequently, it is best to have an electrolytic table. A platinum basin holding not less than 100 c.c. is used. A cylindrical form with flat bottom is convenient. It should rest on a bright copper plate, which is connected with the negative pole of the electrical supply. The positive pole should be also platinum, either a spiral wire, cylinder, or flat foil. Many operators use a funnel-shaped perforated terminal for the negative pole; in which case a glass beaker or casserole will be a suitable container, the positive terminal being placed within the negative.

Four cells of a gravity battery will suffice for a single decomposition, and will operate two, but more slowly. It is usual to arrange the apparatus so that the operation may be continued during the night. When the electricity is taken from the general supply of the laboratory, it is usually necessary to interpose resistance and to have some means of measuring the current-flow. This is sometimes done with

a gas evolution cell and incandescent lamp, but an ammeter and adjustable rheostat are better.

Lactose may be determined by the polarimeter after removal of the fat and proteins, which is best effected, as recommended by Wiley, by acid mercuric nitrate solution. Wiley prepared this by dissolving mercury in twice its weight of nitric acid of 1.42 sp. gr. and adding to the solution five volumes of water, but Revis and Bolton advise that mercuric oxid should be used. The A. O. A. C. optical method is as follows:

For polarimeters reading to 100 for 26.048 grams sucrose (corresponding to 32.98 grams lactose), measure, in c.c., the amount obtained by dividing double this (*i.e.*, 65.96) by the sp. gr., add 10 c.c. mercuric nitrate solution, make up to 102.6 c.c., shake, filter through a dry filter and examine in a 200 mm. tube. Half the observed reading will be the percentage of lactose. For example, if the sp. gr. of the milk is 1.030, the amount taken will be $65.90 \div 1.030 = 64$ c.c.

The allowance for volume of precipitate by making up to 102.6 c.c. is not accurate, except with closely skimmed milks.



FIG. 2.

The correction may be made more closely by calculating the actual volume of the precipitate by multiplying the fat-percentage by 1.075 (average specific volume of fat) and the protein-percentage by 0.8 (average specific volume of coagulated proteins), deducting the sum of these products from 100 c.c. and correcting the observed reading by proportion. For ordinary milk, the volume of the proteins from 65.96 grams may be taken at 1.68 c.c. Supposing the sample to contain 4.0% of fat and the polarimetric reading to be 10, the calculation would be thus:

$$\begin{array}{rcl}
 65.96 \times 0.04 & = & 2.63 \quad \text{Amount of fat in milk taken} \\
 2.63 \times 1.075 & = & 2.82 \text{ c.c. Volume of fat in precipitate} \\
 & & 1.68 \text{ c.c. Est. vol. of proteins in precipitate} \\
 & \text{---} & \\
 & & 4.50 \text{ c.c. Total volume of precipitate} \\
 100 - 4.50 & = & 9.55 \text{ c.c. Actual volume of liquid.} \\
 100 : 95.5 :: 10 : 9.55 & & 9.55 \div 2 = 4.75, \text{ per cent. lactose.}
 \end{array}$$

The employment of a factor for correcting for the volume of precipitate may be avoided by Scheibler's method of "double dilution," in which two solutions of different volume are compared. The following is a summary of the method given by Wiley & Ewell: For polarimeters adapted to a normal weight of 26.048 sucrose, 65.82 grams of milk are placed in a 100 c.c. flask, 10 c.c. of the acid mercuric nitrate

added, the flask filled to the mark, the contents well mixed, filtered, and a reading taken. A similar quantity of the milk is placed in a 200 c.c. flask and treated in the same way. The true reading is obtained by dividing the product of the two readings by their difference. If the observations are made in a 200 mm. tube the percentage is half the true reading.

The instrument should be accurate, and great care taken in the work, or the results will be less satisfactory than by the method first described, in which an allowance is made for the volume of the precipitate.

Multirotation.—When freshly dissolved in cold water, lactose shows a higher rotation than that given above. By standing, or immediately on boiling, the rotary power falls to the point mentioned. In preparing solutions from the solid, therefore, care must be taken to bring them to the boiling-point previous to making up to a definite volume. This precaution is unnecessary when operating on milk.

Acidity.—Milk being often amphoteric to litmus, that indicator cannot be employed in estimating acidity. Phenolphthalein is usually employed. Several methods differing in details have been proposed. Probably the best is that of Thörner. In this, 10 c.c. of milk are diluted with 20 c.c. of water, a few drops of a dilute

alcoholic solution of phenolphthalein added and the titration made with standard alkali. Thörner proposes that the number of c.c. required should be multiplied by 10 and the result termed the "degree of acidity." Fresh normal milk will show figures ranging from 16 to 18. When the degree of acidity is 23 or over, the sample will coagulate on heating.

The process involves a slight error, in that the addition of a notable amount of water to a milk sample disturbs somewhat the relation of the phosphates and diminishes the acidity. It may be advisable to titrate the undiluted milk. If the number of c.c. used is multiplied by 0.9 the lactic acid equivalent to the acidity of the sample is given in grams per 1000 c.c.

DETECTION OF ADULTERATION

By far the larger part of the laboratory work on milk is for assistance in the sanitary control of the supply, and the analyses are principally directed to the detection of the ordinary forms of adulterations. The most important of these are: skimming, watering and use of coloring, thickening and preserving agents. Skimming and watering are detected by determining fat and total solids; from these data the solids not fat are calculated. For the ordinary purposes of milk control, fat can be estimated with quite sufficient accuracy by centrifugal methods. The total solids may be estimated directly as described on page 12, or calculated from the sp. gr. and fat as indicated on page 21.

Judgment whether a given sample has been skimmed or watered depends in many cases upon the standard for whole milk. Some irregularity of standards for fat and solids not fat exists, and the opinion of the analyst will be determined, therefore, by the standard of the locality. In most cases the standard for fat is between 3 and 4%, and that for total solids about 8.50%.

As fat diminishes the sp. gr. of milk, and the

other solids increase it, it is possible to take off a small amount of the former and add some water without disturbing the sp. gr., but, of course, the above analytical methods will detect this procedure. It is now admitted that, except in cases of wide departure from the usual limits, the adulteration of milk cannot be detected by the sp. gr. alone but the employment of a carefully graduated lactometer is of use in routine milk inspection.

Direct Detection of Added Water. Serum-refraction.—Of late years several methods have been proposed for this purpose but most of them have no positive value and have not come into general use. The refractive index of the whey (milk-serum) offers a rapid and satisfactory method for detecting watering. Several methods of preparing this whey have been proposed, but Lythgoe has found, as the result of extended experience, the following to be satisfactory.

Dissolve 7.25 grams of crystallized copper sulfate in water and dilute to 1000 c.c. If this solution does not refract 36 on the scale of the immersion refractometer at 20°, add water or copper sulfate until the desired result is obtained. To 8 c.c. of the copper solution add 32 c.c. of milk. Shake well and pour upon a dry filter. When the filtrate begins to come through clear, change the receiver, pour the small quantity of cloudy filtrate upon

the filter and continue the filtration as usual. Refract the clear filtrate at 20°, by means of the Zeiss immersion refractometer. A reading below 36 indicates added water. The advantages of this method over the acetic acid method are as follows: It is quicker, heating of the samples is unnecessary, consequently there is no error due to evaporation. The range of differences in the refraction of pure milk is less. 10% of added water will reduce the refraction of high-grade milk below the minimum, but it takes 15% in the acetic acid method. Lythgoe made analyses of 150 samples of milk of known purity by this method. The total solids ranged from 17.17 to 10.40%, the fat from 7.7 to 2.45%, the solids not fat from 10.50 to 7.5% and the refraction of the copper serum from 36.1 to 39.5. These refractions were distributed as follows:

REFRACTION	NUMBER OF SAMPLES
39.0 to 39.5	6
38.0 to 38.9	66
37.0 to 37.9	65
36.1 to 36.9	13
	—
	150

See also table of refractions on page 7.

As a result of extended experience, Lythgoe has recently given the following applications of some of the methods of milk analysis.

The least variable constituents of milk are

lactose and ash, both of which are valuable data in detecting added water. It is possible within reasonable limits to indicate by the total solids and fat whether a given sample has been watered or skimmed.

No relation exists between the refraction of the (sweet) serum and the ash of the sour serum (see page 66), therefore, if both these data are below those of normal milk, added water is positively indicated.

The ratio of protein to fat in normal milk is always less than 1. If the ratio exceeds 1, skimming is indicated. If the protein-fat ratio is less than 0.7, or the percentage of fat to total solids is over 35, in samples having a low serum refraction, these may be declared watered, the refraction being not necessarily below the minimum for all samples of known purity.

The sp. gr. of the sweet serum or its total solids may be used as a datum in place of the refraction; either will be a safe guide.

Lowering of Freezing-point.—Several observers have shown that watered milk has a lower freezing-point than pure milk, and that the amount of depression has a definite relation to the amount of water added. One of the most recent statements on the subject is by J. W. Leather, who found the procedure very satisfactory for detecting watering in cows' milk and that of the

India buffalo. He states that one observer has found that a depression to 0.537° indicates 2.3% of added water. The procedure requires special apparatus and careful manipulation; data from testing samples of known composition should be obtained before relying on it in important cases.

Thickening Agents.—To conceal skimming and watering many thickening agents have been used. At least two instances of the use of brain matter have been reported. Dextrin, starch, sugar, salt, gelatin and agar have all been used.

Brain matter can be easily detected by the microscope, starch jelly by the iodine test, dextrin by increased polarimetric reading, sodium chlorid by the increased chlorids in the ash. Agar is frequently used in certain milk products, especially the cheap ice-cream sold in American cities.

Gelatin.—Stokes detects the presence of gelatin in cream or milk as follows: 10 c.c. of the sample, 20 c.c. of cold water, and 10 c.c. of acid mercuric nitrate solution (page 37) are mixed, shaken vigorously, allowed to stand for five minutes, and filtered. If much gelatin is present, it may be difficult to get a clear filtrate. A portion of the filtrate is mixed with an equal bulk of saturated aqueous solution of picric acid.

Gelatin produces a yellow precipitate. Picric acid will detect the presence of 1 part of gelatin in 10,000 parts of water. The picric acid solution should not give a precipitate with the nitrate solution.

For *sucrose* Cotton devised the following tests: 10 c.c. of the sample are mixed with 0.5 gram of powdered ammonium molybdate, and 10 c.c. of dilute hydrochloric acid (1 to 10) are added. In a second tube, 10 c.c. of pure milk or 10 c.c. of a 6% solution of lactose are similarly treated. The tubes are then placed in the water-bath and the temperature gradually raised to about 80°. If sucrose is present, the milk will become blue, while genuine milk or milk-sugar remains unaltered unless the temperature is raised to the boiling-point. According to Cotton, the reaction is well marked in the presence of as little as 1 gram of sucrose to 1000 c.c. of the milk. For the detection of other organic thickening agents, such as pectoses, agar and mixtures of agar and gelatin, see under "Cream," page 67.

Calcium Saccharate (Saccharate of Lime).—A compound produced by the action of lime on sucrose has been used as a thickening agent. A test due to Bauer and Neumann is recommended by Lythgoe, from whose description the following is taken:

To 25 c.c. of milk (or cream) add 10 c.c. of

5% solution of uranium acetate, shake well, allow to stand for five minutes and filter. To 10 c.c. of the clear filtrate (in the case of cream use the total filtrate, which will be less than 10 c.c.) add a mixture of 2 c.c. saturated ammonium molybdate and 8 c.c. dilute hydrochloric acid (1 part 25% acid and 7 parts water), and place in a water-bath at a temperature of 80° for five minutes. If the sample contains sugar the solution will have a prussian blue tint. This should always be compared in a colorimeter with the standard prussian blue solution prepared by adding a few drops of potassium ferrocyanid and 5 drops of 10% hydrochloric acid to a solution of 1 c.c. of 0.1% ferric chlorid in 20 c.c. of water.

It has been claimed that pure milk will give this test. Occasionally samples of pure milk will give a pale blue, but this can be entirely removed by filtration, and the filtrate will be green; while the color due to sucrose will pass through the filter, giving the blue solution characteristic of adulterated samples. The color is due to reduction of molybdic acid, and is caused by levulose and dextrose as well as by sucrose. Solutions of 1 gram of lactose, levulose, dextrose and sucrose in 35 c.c. of water were used in comparing the amount of color produced when heated with the molybdenum reagent for five minutes. Lactose produced no color, levulose gave a heavy

blue, sucrose a weaker blue and dextrose the weakest blue, corresponding in intensity as 10 : 3 : 1.

Stannous chlorid and ferrous sulfate give this color, but the reaction takes place in the cold, and with small quantities the color disappears on heating. In order for the color to persist after heating the sample of cream must contain these substances to the extent of 1% calculated as the metal. In this case the sample will be completely coagulated and the taste will be disagreeable. Hydrogen sulfid will also give the blue, but it will disappear on heating. If the solution does not show blue before heating, it is free from hydrogen sulfid, ferrous sulfate or stannous chlorid.

As a confirmatory test for sugar, the resorcinol test may be applied to the serum prepared with uranium acetate as described. This test is given by sucrose and levulose, but not by dextrose or lactose.

The quantitative estimation of sucrose in milk is given under Milk Products (page 74).

Detection of Heated Milk.—Fresh milk contains one or more enzymes of the “peroxydase” type, that is, having power to bring about transfer of oxygen from peroxids to oxidable substances. As the function of these enzymes is destroyed by temperatures near 100°, it becomes possible to utilize the reaction for deter-

mining whether a given sample has been thus heated. In most cases the action of the enzym is indicated by the production of a deep blue, no color change occurring when the enzym has been heated. Hydrogen peroxid is commonly employed for furnishing the oxygen. A considerable number of substances have been found to be susceptible to oxidation under the influence of the milk enzymes. Benzene derivatives, commonly used as photographic developers are especially susceptible. Guaiacum was first used.

Arnold's Method.—A solution of guaiacum in acetone is, according to Arnold and Menzel better than the ordinary tincture. The test is applied by adding to a small amount of the sample in a test-tube, about 10 drops of the guaiacum solution, to which a drop or two of hydrogen peroxid solution has just been added, so that the reagent will float on the milk. If the sample has not been heated above 80°, the point of contact of the liquids will show a deep blue ring.

As guaiacum is liable to changes both in the solid form and in solution it is important to determine if the reagent is sensitive to raw milk, hence a control test should always be made. Other reagents are now available which are, in the main, more trustworthy.

Dupouy's Method.—In this method, 1-4 diam-inobenzene is used. The reagent is dissolved in

water (a weak solution will suffice), a few drops added to the sample, then a few drops of hydrogen dioxid solution, and the liquids shaken gently. Milk that has not been heated above 80° gives immediately a bright blue. Milk that has been heated above this temperature shows no color change at first but may slowly acquire a bluish tint. This test is much in favor, but it is open to the objection that the solution of the reagent does not keep more than few hours, and even in the solid state some commercial samples soon decompose.

Benzidin Method.—Wilkinson and Peters suggested this reagent, employing a solution of it with a few drops of acetic acid followed as usual by the oxidizing agent. Leffmann finds that the commercial benzidin hydrochlorid (furnished for volumetric estimation of sulfates) acts satisfactorily without acetic acid.

Wilkinson and Peters' test is performed similarly to those just described, and has a similar significance. They give experiments to show that the method is rather more delicate than with diamino-benzene or guaiacum. The solution of the benzidin compound keeps better. They found that milk heated to 77° had lost its reactivity to guaiacum but retained reactivity to the other two reagents. Heated to 78° the reactivity was also lost to these.

Leffmann has found that several commercial photographic developers, *e. g.*, amidol, are applicable in this test with about the limitations above noted.

At critical temperatures, however, the results with all the reagents depend materially on the length of the heating.

Colors.—Annatto, turmeric, and some coal-tar colors are much used. Caramel is occasionally used, saffron and carotin but rarely. *Annatto* may be detected by rendering the sample slightly alkaline by acid sodium carbonate, immersing a slip of filter-paper, and allowing it to remain over night. Annatto will cause a reddish-yellow stain on the paper.

Leys gives the following method for detecting annatto; 50 c.c. of the sample are shaken with 40 c.c. of 95% alcohol, 50 c.c. of ether, 3 c.c. of water, and 1.5 c.c. of ammonium hydroxid solution (sp. gr. 0.900), and allowed to stand for twenty minutes. The lower layer, which in presence of annatto will be greenish-yellow, is tapped off and gradually treated with half its measure of 10% solution of sodium sulfate, the separator being inverted without shaking, after each addition. When the casein separates in flakes that gather at the surface, liquid is tapped off, strained through wire gauze, and placed in four test-tubes. To each of these amyl alcohol is added,

and the tubes shaken and immersed in cold water, which is gradually raised to 80°. The emulsion breaks up, and the alcohol, holding the annatto in solution, comes to the surface. The alcoholic layer is separated from the lower stratum, evaporated to dryness, and the residue dissolved in warm water containing a little alcohol and ammonium hydroxid. Clean white cotton is introduced and the liquid evaporated nearly to dryness on the water-bath. The cotton, which is colored a pale yellow, even with pure milk, is washed and immersed in a solution of citric acid, when it will be immediately red-dened if the milk contains annatto. Saffron, turmeric, and the coloring-matter of the marigold do not give a similar reaction.

Coal-tar colors may often be detected by dyeing wool, but Lythgoe has devised the following method, which is satisfactory: 15 c.c. of the sample are mixed in a porcelain basin with an equal volume of hydrochloric acid (sp. gr. 1.20), and the mass shaken gently so as to break the curd into coarse lumps. If the milk contains an azo-color, the curd will be pink; with normal milk the curd will be white or yellowish.

General Method for Colors in Milk.—Leach devised a general method. 150 c.c. of the sample are coagulated in a porcelain basin, with the addition of acetic acid and heating,

and the curd separated from the whey. The curd will often collect in a mass; but if this does not occur, it must be freed from whey by straining through muslin. The curd is macerated for several hours in a closed flask, with occasional shaking, with ether to extract fat. Annatto will also be removed by it. The ether and curd are separated and treated as follows:

The ether is evaporated, the residue mixed with a little weak solution of sodium hydroxid, and passed through a wet filter; and when this has drained, the fat is washed off and the paper dried. An orange tint shows annatto, which may be confirmed by a drop of solution of stannous chlorid, which makes a pink spot.

If the curd is colorless, no foreign coloring-matter is in it; if orange or brown, it should be shaken with strong hydrochloric acid in a test-tube.

If the mass turns blue gradually, caramel is probably present. The whey should be examined for caramel (see page 95).

If the mass turns pink at once, an azo-color is indicated.

Falsification of the "Cream-line."—The use of glass bottles for retail delivery of milk enables purchasers to make approximate estimations of the richness of the sample by the depth of cream formed after standing for some time, this being

of distinctly different tint from the milk below it. Deception has of late been extensively practised by a treatment of milk which breaks up the fat globules and increases the volume of cream formed, so that a slightly skimmed milk will yield a fair volume of cream. Determination of fat by the usual methods will show the fraud. See page 65.

It has been found that many of the bottles used for distribution of milk are not of the capacity designated on them, but this is a matter of police regulation.

Perservatives.—These are largely used, especially in the warmer season, as a substitute for refrigeration. Many of them are sold under proprietary names which give no indication of their composition. Preparations of boric acid and borax were at one time the most frequent in use, but at present *formalin*, a 40% solution of formaldehyd, has come into favor. Sodium benzoate is now in common use as a preservative of cider, fruit-jellies, and similar articles, and may, therefore, be found in milk. Salicylic acid is not so much employed. Sodium carbonate is occasionally used to prevent coagulation due to slight souring. Fluorids and abrastol may be used. A mixture of boric acid and borax is more efficient than either alone. The quantity generally used is equivalent to about 0.5 gram of

boric acid per 1000 c.c. Formaldehyde is an efficient antiseptic. In the proportion of 0.125 gram to 1000 c.c., it will keep milk sweet for a week. Hydrogen peroxid, ozone and dichromates have been used. The almost universal decree of sanitary authorities is that milk must be free from any added material, but owing to its comparatively high cost, liability to decomposition and the marked characters of even incipient decomposition, great temptation to use preservatives exists and any antiseptic, not actively poisonous, may be used. It has been found that milk drawn and marketed under strict sanitary precautions will keep for a considerable time, even at moderate temperatures. The only permissible method of preserving milk is by refrigeration.

In addition to the descriptions of the detection and estimation of preservatives given below, see also under "Cream."

Formaldehyde. *Hehner's Test.*—Hehner found that when milk containing formaldehyde is mixed with sulfuric acid containing a trace of a ferric compound, a distinct blue appears. Richmond and Boseley showed that the delicacy of the test is much increased if the milk is diluted with an equal volume of water and sulfuric acid of 90 to 94%, added so that it forms a layer underneath the milk. Under

these conditions, milk, in the absence of formaldehyde, gives a slight greenish tinge at the junction of the two liquids, while a violet ring is formed when formaldehyde is present even in so small a quantity as 1 part in 200,000 of milk. The color is permanent for many hours. In the absence of formaldehyde, a brown ring may form in the course of a few hours, but it is below the junction line of the two liquids.

Phenylhydrazin Test.—The following test avoids the fallacy of some other tests. A pinch of phenylhydrazin hydrochlorid is added to a few c.c. of the sample, the liquid shaken, then a drop of a fresh solution of sodium nitroprussid and a few drops of sodium hydroxid solution. A greenish tint is at once produced if formaldehyde is present. If the test is applied to the liquid obtained by distilling milk the color will be deep blue.

Phloroglucol Test.—A small amount of a 1% solution of phloroglucol is added to the sample and then a considerable volume of sodium hydroxid solution. In the presence of formaldehyde a distinct rose tint will be produced. It is best to add the phloroglucol by means of a tube passed to the bottom of the test-tube.

Bonnet's test utilizes the vapor of formaldehyde, and avoids the fallacies of some of the

older tests. A solution is made by dissolving 0.035 gram pure morphin sulfate in 10 c.c. of sulfuric acid. This solution does not keep well. A convenient amount of the sample is placed in a dish or beaker, a watch-glass containing 1 c.c. of the above solution is floated on it, and the dish covered with a glass plate. The materials are allowed to remain undisturbed at room-temperature for several hours. Formaldehyde is indicated by the development of a color ranging from pink to dark blue. A black discoloration is disregarded. Bonnet found that with 1 part of formaldehyde to 25,000 parts of sample a distinct color appeared in one hour.

In testing ice-cream and similar articles it must be borne in mind that some of the flavoring materials being aldehydic in nature may simulate formaldehyde. La Wall has found that vanillin may act thus. The phenylhydrazin and Bonnet tests are least liable to fallacy in this respect.

Nitrites and Formaldehyde.—Mixtures of these substances are now sold under fanciful and misleading names, for milk preservatives as a nitrite prevents the reactions of formaldehyde with some of the tests.

Leffmann has found that the phenylhydrazin test will react promptly with formaldehyde in presence of notable amount of nitrite and also

that the well-known test for nitrites (sulfanilic acid and alphanaphthylamine) reacts in the presence of formaldehyde. The reactions are obtained in fresh samples and in those that have stood for twenty-four hours.

Determination of Formaldehyde.—In the case of milk the proportion of formaldehyde is almost always small and it may be in great part removed from milk by distillation especially in a current of steam. B. H. Smith found that if 100 c.c. of the sample are distilled with 1 c.c. of dilute sulfuric acid (1:3), one-third of the formaldehyde present will come over with the first 20 c.c. Distillation of milk is troublesome owing to bumping, but Smith found that it could be safely conducted with a flat evaporating burner. It is advisable to put a few pieces of pumice into the flask.

Shrewsbury and Knapp recommend the following method for estimation of formaldehyde. An oxidizing reagent is prepared by mixing 0.1 gram of pure nitric acid (sp. gr. 1.52) with 100 c.c. of strong hydrochloric acid are mixed. This mixture should be freshly made.

5 c.c. of milk are treated with 10 c.c. of the reagent, the mixture well shaken and kept for ten minutes in a water-bath at 50°. The depth of color is proportional to the amount of formaldehyde present and by means of milk containing

known amounts of the preservative estimations may be made.

Hydrogen Peroxid.—Many tests have been devised for detection of this substance. Among the most convenient and satisfactory is the reaction with vanadic acid first given by Werther. It may be carried out by adding to 10 c.c. of the milk, 10 drops of a 1% solution of vanadic acid in dilute sulfuric acid. This solution may be conveniently made by dissolved commercial sodium orthovanadate in the dilute acid.

In the presence of hydrogen peroxid a distinct red will appear promptly. Barthel states that a proportion of 0.010 gram of the peroxid in 100 c.c. of milk can be detected positively using only 10 c.c. of the sample.

Benzoates and Salicylates.—The following method covers both these preservatives.

10 c.c. of dilute sulfuric acid (5%) are added to 20 c.c. of 95% alcohol and into this 50 c.c. of the milk are poured in a fine stream with constant stirring. After a few moments, the mixture is filtered, the filtrate being returned until it passes clear. A sufficient volume of the filtrate is extracted in the usual manner with an equal volume of ether or similar solvent. The solvent is divided into two portions that are separately evaporated and tested for benzoic and salicylic acids respectively as given below.

Benzoates.—This is detected by a modification of Mohler's method by Von der Heide and Jakob as given by U. S. Bureau of Chemistry.

The residue that is to be tested for benzoic acid is dissolved in a little water, the solution mixed with from 1 to 3 c.c. of normal sodium hydroxid and evaporated to dryness. To this residue is added from 5 to 10 c.c. of concentrated sulfuric acid and a small crystal of potassium nitrate and the mixture heated either for ten minutes in a glycerol bath between 120° and 130° or for twenty minutes in boiling water. If heated in the glycerol bath the temperature must not be permitted to go over 130°. Metadinitrobenzoic acid is formed. After cooling 1 c.c. of water is added, the liquid made decidedly ammoniacal, boiled to break up ammonium nitrite, and some fresh colorless ammonium sulfid solution added so that the liquids do not mix. A brown ring at junction indicates benzoic acid. The liquids being mixed, the color diffuses and on heating changes to greenish-yellow. The last reaction distinguishes benzoic acid from salicylic and cinnamic acid as these latter form amino-derivatives which are not destroyed by heating. Phenolphthalein interferes with this process.

Salicylic Acid.—The other portion of the ether-extract may be evaporated and tested for

salicylic acid in the usual manner with a ferric compound.

Saccharin.—A suitable amount of the sample (50 or 100 c.c.) is acidified with dilute (25%) sulfuric acid and extracted with a mixture of equal parts of petroleum spirit (boiling below 60°) and ether. The solvent is evaporated at a gentle heat. The presence of saccharin in the residue may be detected by the taste. 2 c.c. of a saturated solution of sodium hydroxid are added and the dish heated until the residue dries and then to 210°–215°, and maintained thus for half an hour. The saccharin is converted into salicylic acid, which may be detected in the residue by acidulating it with sulfuric acid and applying the ferric chlorid test. If salicylic acid be present originally in the sample, the residue from the petroleum spirit and ether solution is dissolved in 50 c.c. of dilute hydrochloric acid, bromin water added in excess, the liquid shaken well, and filtered. Salicylic acid is completely removed as a brominated derivative. The filtrate is made strongly alkaline with sodium hydroxid, evaporated, and fused as described above.

Sodium Carbonate and Sodium Acid Carbonate.—These substances are occasionally added to milk to prevent acidity due to decomposition. Barthel recommends a test devised by Hilger. 50 c.c. of the milk are diluted with 250 c.c. of

water, the mixture is heated, precipitated with a small amount of alcohol and a convenient volume filtered. The filtrate is evaporated to half its bulk. The presence of an alkali-carbonate is easily ascertained by the usual tests.

Borates.—Jenkins' method is convenient and reasonably delicate. 10 c.c. of milk are mixed with 7 c.c. of hydrochloric acid, filtered, a strip of turmeric paper dipped in the filtrate, and then dried on a watch-glass on the water-bath. The paper becomes red in the presence of borates.

A simple test is to mix in a porcelain basin a drop or two of the milk, a drop of hydrochloric acid and a drop of alcoholic solution of turmeric and evaporate to dryness on the water-bath. The residue touched with ammonium hydroxid will show a distinct greenish stain in the presence of very small amounts of borates.

It is obvious that the delicacy of both these tests may be materially increased by concentrating the sample. As boric acid is volatile with steam it is best to render the sample slightly alkaline with sodium hydroxid before evaporating.

Abrastol (Asaprol).—This is a calcium beta-naphthol-sulphonate that has marked antiseptic powers and has been used as a food preservative. The following test suggested by Leffmann will detect very small amounts. 10 c.c. of the sample are mixed with 0.5 c.c. of the solution of

mercuric nitrate described on page 37. In the presence of abrastol a distinct yellow tint is produced in a few minutes. Greater delicacy can be obtained by using the same proportion of the reagent with 10 c.c. of milk known to be pure.

Organic Contamination.—Sanitary control of market-milk also involves tests for animal products, such as pus cells, and the identification of specific microbes, such as those causing tuberculosis and typhoid fever. These investigations, however, are mostly outside of the scope of a work on chemical analysis. For information concerning these recourse must be had to works on pathology and bacteriology.

Several chemical tests have been published by which it is claimed that approximate determination of these contaminating organisms and substances can be made but they are not capable of replacing the exact methods of the pathologic and bacteriologic laboratory. One of these is the following. A dilute solution of methylene blue is prepared by adding 5 c.c. of a saturated alcoholic solution of the dye to 200 c.c. of water. 0.5 c.c. of this solution is added to 10 c.c. of the sample. If the color is discharged promptly, the sample contains over 100,000,000 bacteria per c.c.

Hydrogen dioxid has been shown by the in-

vestigations of Rentschler to kill quickly many forms of microbes, and may be applicable to the purification of milk, when, as in war, systematic protection and inspection are not possible.

Preservation of Samples.—For the preservation of milk samples for a day or two, refrigeration is the best method. Sterilization in the ordinary steam sterilizer used in preparing culture-media, will enable milk to be kept for a considerable time if in a flask closed with a cotton plug. Several preservatives have been proposed for keeping samples. Richmond found small amounts of hydrofluoric acid effective, but it has been but little used. Formaldehyde is very efficient; in large amount it increases the total solids, interferes with the reactions of the proteins and simulates some of the reactions of the carbohydrates. A couple of drops of commercial formalin to 25 c.c. will preserve a sample for several days.

MILK PRODUCTS

CREAM

Cream differs from whole milk principally in the fat-content; the analytic procedures, therefore, follow those indicated under "Milk," except that the high fat may render some modifications advisable. It is better, for instance, to weigh rather than measure cream, and it is often advisable to dilute it with a known weight of water. For the determination of fat the Röse-Gottlieb method is much in favor (see page 72). The following are some special procedures.

Imitation Cream.—By means of special machinery, the fat globules of milk may be broken into very small portions without causing them to coalesce. This is termed "homogenizing" and will give to poor cream an appearance of richness. It is also possible to incorporate butter with skim-milk, producing an article resembling cream. Of course, unsalted, uncolored butter must be used. As butter made in the usual manner, always contains water, the

adulteration may be detected by the change in the refractive power of the serum as described on page 42. H. C. Lythgoe, who has investigated this question, finds that samples adulterated with butter will give a refraction below 36.0. Results may be confirmed by taking the ash of the *sour* serum. A large amount of the sample is taken (as the yield of serum is small), soured with a pure culture of lactic acid bacillus, or with a little sour milk, shaken in a bottle until the fat and curd have separated, the serum drawn off and the ash of 25 c.c. taken. It should not be below 0.73%. The homogenizing of cream without the addition of fat can be detected by microscopic examination.

Formic Acid.—Revis and Bolton state that glucose containing this may be found in cream and give the following method for its detection.

100 grams are diluted with an equal weight of water, 20 c.c. of a 20% solution of phosphoric acid added, and 100 c.c. distilled, the end of the condenser dipping below the surface of milk of lime containing at least 1 gram of calcium hydroxid and 2 c.c. of 3% acetic acid, free from formic. The distillate is evaporated to dryness, sealed in a small tube of hard glass, drawn out at one end that dips into a small U-tube containing 2 c.c. of water, arranged so that none of the water can be drawn into the tube, and heated until

distillation ceases. The water in the U-tube is mixed with 2 c.c. of Schiff's reagent. If formic acid was present, the mixture will become violet within a half hour.

Schiff's reagent is obtained by dissolving 1 gram of rosanilin hydrochlorid in 10 c.c. of water, adding a mixture of 2 c.c. saturated solution of sodium acid sulfite and 0.5 c.c. strong hydrochloric acid, then water to make 100 c.c. The solution keeps for some time in the dark.

Agar.—This is now often used as a thickening agent. Although characteristic diatoms are found in it, the detection of the substance by isolation of these has not been practically successful. Revis and Bolton recommend the following method.

50 grams of the sample are diluted with 100 c.c. of water, heated in boiling water and cleared with 5 c.c. of 10% calcium chlorid solution. The mixture is filtered, preferably in a hot-water funnel, cooled and mixed with about two-thirds its volume of strong alcohol. The precipitate (containing any agar that may have been in the sample) is separated, and boiled with 5 c.c. of water until dissolved. If it contains agar, the solution will gelatinize on cooling. To detect the presence of gelatin in association with agar, the procedure is the same, except that when the precipitate is dissolved, a few c.c. of the solution

are treated with picric acid solution. A precipitate indicates gelatin. In this case, the remainder of the solution is evaporated to small bulk, and mixed with a 10% solution of tannin until no more precipitate is produced. The liquid must in this treatment not have a temperature of over 60°. To it a few c.c of white of egg are added and the mixture heated to boiling for thirty minutes, filtered hot, concentrated to small bulk on the water and allowed to cool and gelatinize.

CONDENSED MILK

Commercial condensed milks present two principal forms, sweetened and unsweetened. In the latter sucrose is generally used. Often constituting more than half the solids of the product. Up to recent years, unsweetened condensed milk was largely sold in the United States as "evaporated cream" but this is now forbidden by the federal food law and by many State enactments.

Dried milk has also been manufactured but does not seem to have met with much favorable reception. Commercial evaporation of milk is conducted at a low temperature so that less modification of the ingredients is produced than in ordinary boiling, but some modification of the lactose may occur which will make polarimetric readings less accurate than with unheated milk.

The analysis of unsweetened condensed milk can be conducted along the same lines as those for ordinary milk and cream, the sample being diluted about three times by adding a known volume of water. It must not be forgotten, that lactose may crystallize from condensed and dried milks, and excessive polarimetric rotation occur in recently made dilutions, unless these are heated to brief boiling and cooled (see page 39). Com-

mercial condensed milks usually represent whole milk concentrated to about one-third or two-sevenths of its original volume. A small amount of invert-sugar may be present. The most common defect in condensed milks is deficiency in fat, due to preparation from closely skimmed milks. Preservatives (other than sucrose) and coloring-matters are rarely used, nor is it likely that foreign fats will be present.

The fat of unsweetened condensed milk can be readily determined by the L-B method (page 18).

In a recent publication, Bigelow and Fitzgerald give the following detailed description of the application of the Leffmann and Beam method to the examination of unsweetened condensed milk:

Weigh 9 grams of evaporated milk into an 8% Babcock milk bottle. Add 10 c.c. of water. Thoroughly mix by shaking and add 3 c.c. of a mixture of equal parts of amyl alcohol and concentrated hydrochloric acid. Shake thoroughly and add 10 c.c. of concentrated sulfuric acid (1.84 sp. gr.) in three or four portions, mixing after each addition. If too much heat develops the bottle may be cooled somewhat in water during the addition of the acid.

Fill the bottle to near the base of the neck with a hot fresh mixture of equal parts of sulfuric acid and water. Thoroughly mix the contents

of the bottle by shaking. Raise the fat column to the top of the scale by means of the acid and water mixture, and whirl for five minutes. Read promptly (see page 20) from the extreme bottom of the fat column to the bottom of the upper meniscus. Multiply the reading by 2, and deduct 0.25; the remainder is the per cent. of fat.

If an electric centrifuge without heat has been employed, the fat column will be somewhat cool and should be heated, before reading, in a water-bath about 60°.

The same authors give the opinion that the centrifugal methods are not sufficiently accurate to be depended upon for determining if evaporated milk is up to standard. The Röse-Gottlieb method is best for this purpose. If the centrifugal methods are employed, considerable allowance must be made for inaccuracies. Results obtained are inaccurate unless the fat column is clear, with the meniscus at the bottom of the column perfect and not distorted by either char or milky appearance.

The percentage of solids as calculated from the sp. gr. is not sufficiently accurate to determine whether the milk complies with the standard unless the correction factor for the formula of calculation is ascertained frequently by the determination of solids by drying.

The full analysis of *sweetened condensed milk* is difficult, and many of the published figures are erroneous. The sucrose interferes with the extraction of the fat by solvents. The same difficulty occurs in the analysis of some prepared infant-foods, such as mixtures of milk with malt and glucose.

For the general operations, a portion of the well-mixed contents of a freshly opened can should be accurately weighed, diluted with a known amount of water, and well mixed, from which mass the portions for analysis may be taken and the results calculated to the original sample. 50 grams mixed with 150 c.c. of water will be a convenient quantity. For the polarimetric determination of lactose, a special procedure will be necessary; but for determination of solids, ash, total proteins, and total reducing sugars, the examination may be made as with ordinary milk upon this diluted sample.

Fat.—The Adams method is not satisfactory under ordinary conditions, owing to the sucrose. The Röse-Gottlieb method is now largely used and generally approved. The following description is given by Bigelow and Fitzgerald:

Weigh from 4.5 to 5.0 grams evaporated or condensed milk into a Röse-Gottlieb tube, add water to make about 11 grams and $1\frac{1}{4}$ to $1\frac{1}{2}$

c.c. concentrated ammonium hydroxid, and thoroughly mix by shaking.

Add 10 c.c. 95 % alcohol and shake thoroughly. Fill up to the level of the side tube with water, if necessary, and shake. Add 25 c.c. ether and shake well for one minute. Add 25 c.c. petroleum spirit (b. p. below 65°) and shake well for one minute.

Allow tube to stand until layers separate well. Draw off ether-fat solution as completely as practicable and run it through a small quick-acting filter into a weighted flask (weighted by counterpoising, if the test is not finished the day it is started.)

Re-extract the liquid in tube as before with 15 c.c. of each of petroleum spirit and ether, shaking after each is added. Before the addition a little alcohol may be added and the contents of the tube mixed by shaking, to bring the layer of ammoniacal liquid close up to the outlet tube, for by repeated extractions the surface of separation is lowered.

Run the solution from the second extraction through the filter into the flask, and wash end of spigot, filter paper, and lower surface of the funnel with ether; or, better, with a mixture of equal parts of ether and petroleum spirit which has been allowed to stand for separation of water.

In the examination of cream a third extraction is necessary, but with evaporated and condensed milk the third extraction does not recover more than 0.02 or 0.03 % of fat, and may be omitted.

Evaporate the liquid slowly on a steam bath and dry the fat in steam oven until its weight is constant. Weigh after one hour and then at half-hour intervals. As soon as the fat begins to gain in weight stop drying and take the next previous weight. Increase of weight is due to oxidation after all moisture and alcohol are gone. In all cases the drying should be completed the day it is begun.

Double Extraction.—The following is given as a provisional A. O. A. C. method: Extract with ether, as usual, about 5 grams of a 40% solution, dry, leave the tubes in a dish containing at least 500 c.c. of water, dry, extract again with ether for four hours.

Sugars.—If regard is to be given to the presence of invert-sugar, a special method must be followed. The processes first given consider lactose and sucrose only. The heating employed in the manufacture of condensed milk may reduce the rotatory power of lactose sufficiently to cause error in the polarimetric method. The reducing power with alkaline copper solutions is not seriously affected.

Determination of sucrose may be made by

difference; that is, subtracting the sum of the other ingredients from the total solids. This will serve for ordinary inspection purposes, since the amount present is almost always large, generally more than the total of milk-solids, and a slight error does not affect the judgment as to the wholesomeness of the sample. Exact work requires, however, that the sucrose be determined directly. Several processes have been devised for the purpose. Sucrose exerts but little action on Fehling's solution, but invert-sugar acts powerfully, and some processes depend on determining the reducing power before and after inversion. Since the polarimetric reading is also markedly changed by the inversion, the difference in polarization may be employed. Fermentation may be so conducted as to remove the sucrose (also any form of glucose) while the lactose is unaffected. This method is chiefly valuable for recognizing invert-sugar or either of its constituents.

Inversion Methods.—These must be such as to secure prompt inversion of the sucrose without affecting the lactose. Experiment shows that citric acid and invertase are the most suitable agents. Stokes & Bodmer have worked out the citric acid method substantially as follows:

25 c.c. of the diluted sample are coagulated by addition of 1% of citric acid, without heating,

and made up to 200 c.c. plus the volume of the precipitated fat and proteins (see page 38). The liquid portion, which now measures 200 c.c., is passed through a dry filter. The reducing power with alkaline copper solutions is determined at once upon 50 c.c. of this filtrate. To another 50 c.c., 1% of citric acid is added, the solution boiled at least thirty minutes, and the reducing power also determined. The increase over that of the first solution is due to the invert-sugar formed by the action of the citric acid on the sucrose. It is necessary to bear in mind that the reducing equivalents of lactose and invert-sugar are not the same. Volumetric methods may be employed.

The following method is based on the difference in polarimetric reading before and after action of invertase. 75 c.c. of the diluted milk are placed in a 100-c.c. flask, diluted to about 80 c.c., heated to boiling, to correct birotation, cooled, and 10 c.c. of acid mercuric nitrate solution (page 37) added. The mixture is made up to 100 c.c., well shaken, filtered through a dry filter, and the polarimetric reading taken at once. It will be the sum of the effect of the two sugars. The volume of the sugar-containing liquid is calculated by allowing for the precipitated proteins and fat, as described on page 38.

50 c.c. of the filtrate are placed in a flask

marked at 55 c.c., a piece of litmus paper dropped in, and the excess of nitric acid cautiously neutralized by sodium hydroxid solution. The liquid is then faintly acidified by a single drop of acetic acid (it must not be alkaline), a few drops of an alcoholic solution of thymol are added, and then 2 c.c. of a solution of invertase, prepared by grinding half a cake of ordinary compressed yeast with 10 c.c. of water and filtering. The flask is corked and allowed to remain at a temperature of 35° to 40° for twenty-four hours. The cane-sugar will be inverted, while the milk-sugar will be unaffected. The flask is filled to the mark (55 c.c.) with washed aluminum hydroxid and water, mixed, filtered, and the polarimetric reading taken. The amount of cane-sugar can be determined from the difference in the two readings by the formula

$$S = \frac{100a + b}{142.68 - \frac{t}{2}}$$

in which S is the percentage of sucrose; a , the reading before, b , after inversion; t , the temperature.

LACTOSE, SUCROSE AND INVERT-SUGAR.—Bige-low and McElroy propose the following routine method to include invert-sugar. The reagents are:

Acid Mercuric Iodid.—Mercuric chlorid, 1.35 grams; potassium iodid, 3.32 grams; glacial acetic acid, 2 c.c.; water, 64 c.c.

Alumina-cream.—A cold saturated solution of alum is divided into two unequal portions, a slight excess of ammonium hydroxid is added to the larger portion and the remainder added until a faintly acid reaction to litmus is obtained.

The entire contents of the can are transferred to a porcelain dish and thoroughly mixed. A number of portions of about 25 grams are weighed carefully in 100 c.c. flasks. Water is added to two of the portions, and the solutions boiled. The flasks are then cooled, clarified by means of a small amount of the acid mercuric iodid and alumina cream, made up to mark, filtered, and the polarimetric reading noted. Other portions of the milk are heated in the water-bath to 55°; one-half of a cake of compressed yeast is added to each flask and the temperature maintained at 55° for five hours. Acid mercuric iodid and alumina-cream are then added, the solution cooled to room temperature, made up to mark, mixed, filtered, and polarized. The amount of sucrose is determined by formula given above. Correction for the volume of precipitated solids may be made by the double-dilution method. The total reducing sugar is estimated in one of the portions by one of

the reducing methods, and if the sum of it and the amount of sucrose obtained by inversion is equal to that obtained by the direct reading of both sugars before inversion, no invert-sugar is present. If the amount of reducing sugar seems to be too great, the lactose must be re-determined as follows: 250 grams of the condensed milk are dissolved in water, the solution boiled, cooled to 80° , a solution of about 4 grams of glacial phosphoric acid added, the mixture kept at 80° for a few minutes, then cooled to room temperature, made up to mark, shaken, and filtered. It may be assumed that the volume of the precipitate is equal to that obtained by mercuric iodid solution. Enough sodium hydroxid is then added to not quite neutralize the free acid, and sufficient water to make up for the volume of the solids precipitated by the phosphoric acid. The mixture is then filtered and the filtrate is measured in portions of 100 c.c. into 200-c.c. flasks. A solution containing 20 milligrams of potassium fluorid and half a cake of compressed yeast is added to each flask, and the mixture allowed to stand for ten days at a temperature between 25° and 30° . Invert-sugar and sucrose are fermented and removed by the yeast in the presence of a fluorid; lactose is unaffected. The flasks are filled to the mark and the lactose determined either by reducing or by the polariscope. The amount

of copper solution reduced by the lactose and invert-sugar, less the equivalent of lactose remaining after fermentation, is due to invert-sugar.

BUTTER

Butter is a mixture of fat, water, and curd. The water contains lactose and the salts of the milk. Common salt is usually present, being added after the churning. Artificial coloring is frequently used.

Butter-fat is distinguished from other animal fats in that it contains a notable proportion of acid radicles with a small number of carbon atoms. Thus, about 91% consists of palmitin and olein and the remainder of butyric and caproic, along with small amounts of caprylic, caprin, myristin, and some others. According to the experiments of Hehner & Mitchell, stearin is present only in very small quantity. The exact arrangement of the constituents is unknown.

The composition of good commercial butter usually ranges within the following limits:

Fat.....	78% to 94%
Curd.....	1% to 3%
Water.....	5% to 14%
Salt.....	0% to 7%

Butter containing over 40% of water is sometimes sold. Such samples are pale and spongy, lose weight, and become rancid rapidly.

The official methods of the A. O. A. C. for the analysis of butter are as follows:

Preparation of the Sample.—If large quantities of butter are to be sampled, a butter trier or sampler may be used. The portions thus drawn, about 500 grams, are to be perfectly melted in a closed vessel at as low a temperature as possible, and when melted the whole is to be shaken violently for some minutes until the mass is homogeneous and sufficiently solidified to prevent the separation of the water and fat. A portion is then poured into the vessel from which it is to be weighed for analysis, and should nearly or quite fill it. This sample should be kept in a cold place until analyzed.

WATER.—From 1.5 to 2.5 grams are dried to constant weight at the temperature of boiling water, in a dish with flat bottom, having a surface of at least 20 sq. cm. The use of clean dry sand or asbestos with the butter is admissible, and is necessary if a dish with round bottom be employed.

FAT.—The dry butter from the water determination is dissolved in the dish with absolute ether. The contents of the dish are then transferred to a weighed Gooch crucible with the aid of a wash-bottle filled with the solvent, and are washed until free from fat. The crucible and

contents are heated at the temperature of boiling water till the weight is constant.

The fat may also be determined by drying the butter on asbestos or sand, and extracting by anhydrous alcohol-free ether. After evaporation of the ether the extract is heated to constant weight at the temperature of boiling water and weighed.

CASEIN, ASH, CHLORIN.—The crucible containing the residue from the fat determination is covered and heated, gently at first, gradually raising the temperature to just below redness. The cover is removed and the heat continued until the material is white. The loss in weight represents casein, and the residue mineral matter. In this mineral matter dissolved in water slightly acidulated with nitric acid, chlorin may be determined gravimetrically with silver nitrate, or, after neutralization with calcium carbonate, volumetrically, using potassium chromate as indicator.

SALT.—About 10 grams are weighed in a beaker in portions of about 1 gram at a time taken from different parts of the sample. Hot water (about 20 c.c.) is now added to the beaker, and after the butter has melted, the mass is poured into the bulb of a separating funnel, which is then closed and shaken for a few moments. After standing until the fat has all collected, the water

is allowed to run into an Erlenmeyer flask, with care not to let fat globules pass. Hot water is again added to the beaker, and the extraction is repeated from ten to fifteen times, using each time from 10 to 20 c.c. of water. The resulting washings contain all but a mere trace of the salt originally present in the butter. The chlorin is determined volumetrically in the filtrate by means of standard silver nitrate and potassium chromate indicator and calculated to sodium chlorid.

Butter-substitutes.—The chief adulteration of butter consists in the substitution of foreign fats, especially the product known as oleomargarin.

When fats are saponified and the soap treated with acid, the individual fatty acids are obtained. It is upon the recognition of the peculiar acid radicles existing in butter that the most satisfactory method of distinguishing it from other fats is based. Since the relative proportion of these radicles differs in different samples, the quantitative estimation cannot be made with accuracy; but when the foreign fats are substituted to the extent of 20% or more, the adulteration can be detected with certainty and an approximate quantitative determination made.

The detection of adulteration of butter-fat by other fats is generally carried out by the deter-

mination of the volatile acid, but some other confirmatory processes are occasionally employed.

QUALITATIVE TESTS.—Two tests are convenient for preliminary examinations, especially for sorting out, when many samples are to be tested. The experience of Dr. William Beam and myself in testing many hundred samples for the Dairy and Food Commissioner of Pennsylvania showed that the methods are satisfactory and useful.

Heating test.—When butter is heated in a small tin dish directly over a gas flame, it melts quietly, foams, and may run over the dish. Oleomargarin, under the same conditions, sputters noisily as soon as heated and foams but little. Even mixtures of butter and other fats show this sputtering action to a considerable extent. The test is not applicable to butter which has been melted and reworked (renovated or process butter).

Saponification test.—An alcoholic solution of sodium hydroxid, boiled up with butter, and then emptied into cold water, gives a distinct odor of pineapples, while oleomargarin gives only the alcoholic odor.

QUANTITATIVE METHODS. *Volatile Acids.*—This method, suggested by Hehner & Angell, systematized by Reichert, is generally called the Reichert process. In this form it is carried out by saponifying 2.5 grams of the fat, adding

excess of sulfuric acid, distilling a definite portion of the liquid, and titrating the distillate with $N/_{10}$ alkali. The number of c.c. of this solution required to overcome the acidity of the distillate is called the *Reichert number*. E. Meissl suggested the use of 5 grams, and the number so obtained is called the *Reichert-Meissl number*. Alcoholic solution of potassium hydroxid was originally used for saponification, but the solution devised by Leffmann & Beam, namely, sodium hydroxid in glycerol, is more satisfactory. This procedure is now official in the U. S. and several European countries. The reagents and operation are as follows:

Glycerol-soda.—100 grams of good sodium hydroxid are dissolved in 100 c.c. of distilled water and allowed to stand until clear. 20 c.c. of this solution are mixed with 180 c.c. of pure concentrated glycerol. The mixture can be conveniently kept in a capped bottle holding a 10-c.c. pipet, with a wide outlet.

Sulfuric Acid.—20 c.c. of pure concentrated sulfuric acid, made up with distilled water to 100 c.c.

Sodium Hydroxid.—An approximately $N/_{10}$, accurately standardized, solution of sodium hydroxid.

Indicator.—Solution of phenolphthalein.

A 300-c.c. flask is washed thoroughly, rinsed

with alcohol and then with ether, and thoroughly dried by heating in the water-oven. After cooling, it is allowed to stand for about fifteen minutes and weighed. (In ordinary operation this preparation of the flask may be omitted.) A pipet, graduated to 5.75 c.c., is heated to about 60° and filled to the mark with the well-mixed fat, which is then run into the flask. After standing for about fifteen minutes the flask and contents are weighed. 20 c.c. of the glycerol-soda are added and the flask heated over the Bunsen burner. The mixture may foam somewhat; this may be controlled, and the operation hastened by shaking the flask. When all the water has been driven off, the liquid will cease to boil, and if the heat and agitation be continued for a few moments, complete saponification will be effected, the mass becoming clear. The whole operation, exclusive of weighing the fat, requires about five minutes. The flask is withdrawn from the heat and the soap dissolved in 135 c.c. of water. The first portions of water should be added drop by drop, and the flask shaken between each addition in order to avoid foaming. When the soap is dissolved, 5 c.c. of the dilute sulfuric acid are added, a piece of pumice dropped in (this must not be omitted), and the liquid distilled until 110 c.c. have been collected. The condensing tube should be of glass, and the distilla-

tion conducted at such a rate that the above amount of distillate is collected in thirty minutes.

The distillate is usually clear; if not, it should be thoroughly mixed, filtered through a dry filter, and 100 c.c. of the filtrate taken. A little of the indicator is added to the distillate, and the standard alkali run in from a buret until neutrali-

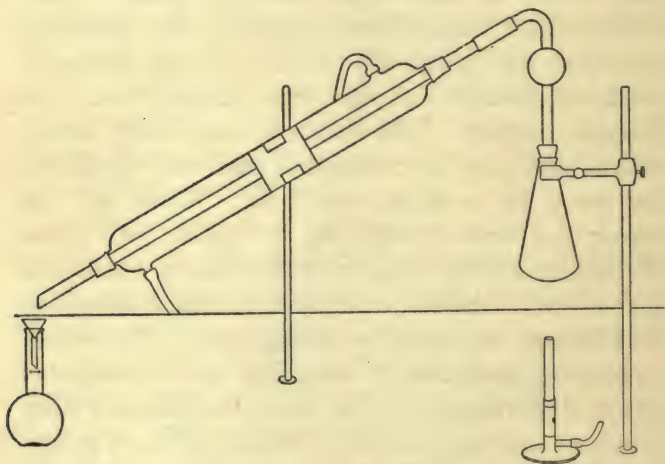


FIG. 3.

zation is attained. If only 100 c.c. of the distillate have been used for the titration, the c.c. of alkali used should be increased by one-tenth.

The distilling apparatus shown in figure 3 is that recommended by the A. O. A. C. (and since adopted in Great Britain), and the directions for

preparing the flask are also from the same source.

When it is intended merely to distinguish butter from oleomargarin, it will be sufficient to saponify 3 c.c. of the clarified fat, dilute, acidify, distil 100 c.c. in the ordinary manner and titrate as directed. "Straight oleos," that is, samples containing inappreciable amounts of butter, will give a distillate requiring only a few c.c. of alkali.

Butter (5 grams) yields a distillate requiring from 24 to 34 c.c. of $N/_{10}$ alkali. Several instances have been published in which genuine butter has given a figure as low as 22.5 c.c., but such results are uncommon. The materials employed in the preparation of oleomargarin yield a distillate requiring less than 1 c.c. of alkali. Commercial oleomargarin is usually churned with milk in order to secure a butter flavor, and, thus acquiring a small amount of butter-fat, yields distillates capable of neutralizing from 1 to 2 c.c. of alkali.

If coconut oil has been used in the preparation of the oleomargarin, the figure will be higher, but there will still be no difficulty in distinguishing pure butter.

The determination of the Reichert number will usually give sufficient information as to the nature of a butter sample. In doubtful cases it may be of advantage to apply other tests as corroborative evidence.

Index of Refraction.—This datum differs notably in different oils, but it is not of much value in detecting adulteration unless considerable of the adulterant be present. Several instruments have been devised for making refraction determination; a familiar one is the butyrorefractometer of Zeiss.

The butyrorefractometer has been strongly recommended for the examination of butter. It is equally adapted for the general examination of fats and oils, and may be used for the determination of the index of refraction as well. As these instruments are made by only one firm and are furnished with directions for use, further description will not be required.

Renovated Butter.—So-called “process” or “renovated” butter, made by melting old or inferior samples, purifying the fat, coloring and salting, is now a familiar article. When heated in a dish such butter sputters, with but little foaming as does oleomargarin, but yields with alcoholic solution of sodium hydroxid the pineapple odor. The fat or process butter gives refractometric data and Reichert-Meissl data similar to ordinary butter. Hess and Doolittle state that the curd of process butter has characteristic qualities, and propose the following method for detecting it.

50 grams of the sample are melted in a beaker at about 50°. Ordinary butter yields a clear

fat almost as soon as melted, while with process butter the fat may remain turbid for a long while. When the curd has largely settled, as much of the fat is poured off as possible, and the remaining mixture is thrown on a wet filter, by which the water will drain away, carrying the soluble proteins and salt. A few drops of acetic acid are added to the filtrate and the mixture is boiled. The filtrate from ordinary butter gives a slight milkiness, but that from process butter gives a flocculent precipitate. Quantitative examination is made by dissolving 50 grams of the sample in ether; if it is ordinary butter, the curd is so finely divided that it remains suspended for some time. As much as possible of the solution is decanted and the mass transferred to a separator, the casein, water, and salt removed, and the remainder washed three times, at least, with ether to remove the fat. The curd is collected on a filter, washed with water, and the nitrogen determined by treating the precipitate with the filter by the Kjeldahl-Gunning method. The filtrate from the curd is made slightly acid with acetic acid, boiled, the precipitated proteins collected on a filter, and the total nitrogen determined. The factor 6.38 may be used in each case for converting the nitrogen into proteins.

A distinction between ordinary and process butter may often be made by microscopic ex-

amination under polarized light with crossed nicols (*i. e.*, dark field), when the process butter appears mottled, owing to the presence of crystals.

Butter Colors.—Butter and butter-substitutes are usually artificially colored. Turmeric and annatto or azo-colors allied to methyl-orange are used.

Azo-colors.—These may be detected by the test devised by Geisler. A small amount of the sample, or, better, the fat filtered from it, is mixed on a porcelain plate with a little fullers' earth. Azo-colors give promptly a red mass; if they are not present, the mixture becomes only yellow or light brown. All samples of fullers' earth are not equally active, and tests should be made with different samples by using fat known to contain the azo-compound until a good specimen of the earth is secured.

For the detection of very minute quantities of the color, the sample may be dissolved in light petroleum, and the fullers' earth added to the solution, when the pink color will appear as a distinct ring or zone at the edge of the deposited layer of the reagent.

Low has proposed the following test for the yellow azo-color: A few c.c. of the filtered fat are mixed in a large test-tube with an equal volume of a mixture of one part strong

sulfuric acid and four parts glacial acetic acid. The contents of the tube are then heated almost to boiling and thoroughly mixed by violently agitating the bottom of the tube. When now allowed to stand and separate, the lower layer of mixed acids will be strongly colored wine-red if the azo-color be present. Pure butter-fat imparts no color to the acids, or, at most, only a faint brownish tinge.

Turmeric and Annatto.—Martin's test will usually be satisfactory: 2 c.c. carbon disulfid are mixed with 15 c.c. of alcohol, by adding small portions of the disulfid to the alcohol and shaking gently; 5 grams of the butter-fat are added to this mixture in a test-tube and shaken. The disulfid falls to the bottom of the tube, carrying with it the fatty matter, while any artificial coloring-matter remains in the alcohol. The separation takes place in from one to three minutes. If the amount of the coloring-matter is small, more of the fat may be used. If the alcoholic solution be evaporated to dryness and the residue treated with concentrated sulfuric acid, annatto will be indicated by the production of a greenish-blue color. With many samples of oleomargarin, a pink tint will be produced, which indicates an azo-color.

Palm oil has been used as a coloring agent in butter-substitutes. Crampton & Simons have

found that two tests devised for detection of rosin-oil can be satisfactorily adapted to detection of palm oil. Success depends on several points. The sample must be kept in a cool dark place until used, filtered at a temperature not above 70° , the heating as brief as possible, and promptly tested. The reagents must be pure and colorless. Cochran finds that annatto will simulate palm oil in these tests, and hence the absence of the former must be assured (see above) before inferring the presence of the latter.

Halphen method.—100 c.c. of the filtered fat are dissolved in 300 c.c. petroleum spirit and shaken out with 50 c.c. of potassium hydroxid solution (0.5 % of hydroxid). The water is drawn off, made distinctly acid with hydrochloric acid, and shaken out with 10 c.c. of carbon tetrachlorid. This solution is drawn off, and part of it tested by adding to it 2 c.c. of a mixture of 1 part crystallized phenol in 2 parts carbon tetrachlorid. To this add 5 drops of hydrobromic acid (sp. gr. 1.19). The test is best performed in a porcelain basin and the contents mixed by agitating gently. Palm oil gives almost immediately a bluish-green liquid.

Liebermann-Storch method.—10 c.c. of the filtered fat are shaken with an equal volume of acetic anhydrid, one drop of sulfuric acid (sp. gr. 1.53) is added and the mixture shaken for

a few seconds. If palm oil be present, the heavier layer separating will be blue with a tint of green.

Egg-yolk has been proposed as a color for oleomargarin, and although its use is unlikely, the possibility of it should be borne in mind. To detect it, about 10 grams of the filtered fat should be shaken with warm alcohol, the liquid drawn off as closely as possible and evaporated to dryness. The coloring matter of egg-yolk is soluble in alcohol, but insoluble in water. It may be distinguished from turmeric by moistening it with a few drops of a mixture of boric and hydrochloric acids, and drying at a gentle heat. Turmeric becomes brown; egg-color is not affected. Egg-yolk contains considerable lecithin, a phosphoric acid derivative. Pure fats contain no phosphorus compound. If, therefore, a few grams of the fat, carefully freed from water or curd, are charred and the mass extracted by boiling with nitric acid, the filtered solution should not give an appreciable precipitate with ammonium molybdate.

Vegetable colors may be detected by boiling up the filtered fat with water, drawing off the watery liquid, adding a few drops of hydrochloric acid and heating the mixture with a piece of clean, undyed wool. True butter colors will not dye wool under these circumstances.

Caramel may be detected by shaking the

watery solution with fuller's earth and filtering. The filtrate is notably paler if caramel is present. Fuller's earth differs in efficiency, and each sample should be tested on known solutions.

Preservatives.—The preservatives used in milk may be found in limited amount in butter, but a mixture of boric acid and borax is often added as a substitute for salt.

Glucose is sometimes used as a preservative, especially in butter intended for export to tropical countries. Crampton found as much as 10% in a sample of highly colored butter intended for exportation to Guadeloupe. For the detection of glucose the phenylhydrazin test might be used. For determination Crampton used the following method: 10 grams of the sample were washed with successive portions of convenient bulk, the solution made up to 250 c.c., and an aliquot portion determined, as given for lactose on page 32. The solution may also be clarified by alumina-cream or acid mercuric nitrate and examined in the polarimeter.

Boric Acid.—25 grams of the sample are melted, the watery portion separated and tested as described on page 62.

CHEESE

Cheese is the curd of milk which has been separated from it, pressed, and undergone some fermentation. The precipitation is produced either by allowing the milk to become sour—when the lactic acid is the agent—or by rennet. The first-named method is mainly applied to the manufacture of so-called Dutch or sour-milk cheese, green Swiss cheese, and cottage cheese. More commonly cheese is obtained by means of rennet derived from the fourth stomach of the calf. The action is due to an enzym which acts directly on the proteins and does not produce its affect through the intervention of acids. The curd (cheese) undergoes, by keeping, various decompositions, some essentially putrefactive, and due to the action of microbes. The decomposition of the cheese is termed “ripening.”

In the sour milk cheeses, ripening is restricted intentionally, since there is liability to an irregular and miscellaneous bacterial growth by which the fermentations may be carried too far, undesirable and even harmful products being formed. Such cheeses are intended for prompt use.

Cheese contains no casein, if by this term

is meant the protein as it exists in milk, or as precipitated from milk by acids. When milk is coagulated by rennet, only a part of the proteins enter into the curd; true casein contains about 15.7% of nitrogen, but the protein matter of cheese contains about 14.3%. Under the process of ripening this is further decomposed, amino- and ammonium compounds, peptones and albumoses being formed.

The following figures, obtained by Van Slyke, will serve to give some idea of the extent to which the curd is changed in ripening. The figures represent average percentage on the total nitrogen. The cheese was an American cheddar:

	GREEN CHEESE	AFTER FIVE MONTHS
Soluble nitrogen compounds...	4.23	35.52
Soluble amino compounds.....	none	11.66
Soluble ammonium compounds	none	2.92

Van Slyke's experiments seem also to indicate that the cheese ripened more rapidly when the curd was precipitated by a larger quantity of rennet and, especially, that cheese rich in fat ripened more rapidly than skim-milk cheese.

In addition to the fat and nitrogenous compounds just mentioned, cheese may contain a small amount of lactose and of lactic and other organic acids. There is present also a certain proportion of mineral matter, alkaline and earthy

phosphates, along with any salt that has been added. Traces of nitrates have been found.

Skimmed milk is not infrequently used for the production of cheese. Partially-skimmed milk is used in the preparation of certain Dutch cheeses. Foreign fats, such as are used in the manufacture of oleomargarin, are sometimes incorporated, the article being known as "filled cheese."

The common American cheese is known as Cheddar. According to Van Slyke, this has, when ripe, the following average composition:

Water.....	31.50%
Fat.....	37.00%
Proteins.....	26.25%
Ash, sugar, etc.....	5.25%

The ash of cheese consists largely of calcium phosphate and salt. Mariani & Tasselli determined the total ash, chlorin, calcium, and phosphoric acid in 15 samples of cheese. The amounts of salts (calculated from the chlorin) depend on the mode of salting. The proportion of phosphoric oxid was always greater than that necessary to form tricalcium phosphate, ranging from 1.07 and 1.08 equivalents of phosphoric anhydrid to calcium oxid in cheese made from sour milk to 1.56 to 1 in Gorgonzola, 1.67 to 1 in skim-milk cheese, and 1.75 to 1 in Edam cheese. The largest quantities of calcium and phosphoric oxid were found in sheep's-milk cheese and in

cheese made from sour milk, whence it follows that acidity does not prevent the precipitation of calcium phosphate in the curds. The excess of phosphoric oxid obtained was attributed to acid phosphates.

The salt in cheese usually ranges between 1 and 4%.

Analytic Methods.—The analytic points usually determined in regard to cheese are water, fat, casein, ash, the presence of fats other than butter-fat, and coloring-matters.

In addition to this, especially in comparing the qualities of genuine cheeses, the proportion of proteic, aminic, and ammoniacal nitrogen is of value.

Care should be taken to select for analysis a sample which represents the average composition of the entire cheese.

The following methods for the determination of water, fat, ash, total nitrogen, and acidity have been adopted by the A. O. A. C.:

Sampling.—When the cheese can be cut, a narrow wedge-shaped segment, reaching from the outer edge to the center of the cheese, is taken. This is to be cut into strips and passed through a sausage-grinding machine three times. When the cheese cannot be cut, samples are taken by a cheese trier. If only one plug can be obtained, this should be perpendicular to the

surface, at a point one-third of the distance from the edge to the center of the cheese. The plug should reach entirely through, or only half-way through, the cheese. When possible, draw three plugs—one from the center, one from a point near the outer edge, and one from a point half-way between the other two. For inspection purposes, the rind may be rejected; but for investigations requiring the absolute amount of fat in the cheese, the rind is included in the sample. It is preferable to grind the plugs in a sausage machine, but when this is not done, they should be cut very fine and carefully mixed.

Water.—Between 2 and 5 grams of the sample should be placed in a weighed platinum or porcelain dish which contains a small amount of material, such as freshly ignited asbestos or sand, to absorb the fat that may run out. This is then heated in a water-oven for ten hours and weighed; the loss in weight is considered as water. If preferred, the dish may be placed in a desiccator over concentrated sulfuric acid and dried to constant weight, but this may require many days. The acid should be renewed when the cheese has become nearly dry.

Fat.—The extraction-tube described on page 16 is prepared as follows: The perforations in the bottom of the tube are covered with asbestos,

on which is placed a mixture containing equal parts of anhydrous copper sulfate and pure dry sand to the depth of about 5 cm., packed loosely, and the upper surface covered with a film of asbestos. On this are placed from 2 to 5 grams of the sample, the mass extracted for five hours with anhydrous ether, then removed and ground to fine powder with pure sand in a mortar. The mixture is replaced in the extraction tube, the mortar washed free from all matters with ether, the washings being added to the tube, and the extraction is continued for ten hours. The fat so obtained is dried at 100° to constant weight.

Here, as in most extractions, carbon tetrachlorid can be substituted for ether, but the results obtained are not necessarily equivalent, and in official analyses the official method must be used.

Total Nitrogen.—This is determined by the Kjeldahl-Gunning method, using 2 grams of the sample. The percentage, multiplied by 6.38, gives the nitrogen compounds.

Ash.—The dry residue from the water determination may be taken for the ash. If the cheese is rich, the asbestos will be saturated therewith. This mass may be ignited carefully, and the fat allowed to burn off, the asbestos acting as a wick. No extra heating should be applied during the operation, as there is danger of spurt-

ing. When the flame has died out, the burning may be completed in a muffle at low redness. When desired, the salt may be determined in the ash by titration with silver nitrate and potassium chromate.

Provisional Method for the Determination of the Acidity on Cheese.—Water at a temperature of 40° is added to 10 grams of finely divided cheese until the volume equals 105 c.c., agitated vigorously, and filtered. Portions of 25 c.c. of the filtrate corresponding to 2.5 grams of the cheese are titrated with decinormal solution of sodium hydroxid, using phenolphthalein as indicator. The amount of acid is expressed as lactic acid.

The above processes may be advantageously modified in some respects. The determination of water may be made by the extraction of the cheese with alcohol and ether and drying of the alcohol-ether extract and fat-free solids separately. Blyth recommends this method as more accurate and less tedious than the direct drying. In the determination of ash it will be better to extract the charred mass with water and proceed as described in the determination of the ash of milk.

The fat extracted by ether may be examined for other than butter-fat by the distillation method in the usual way. When the composition of the fat is alone desired, it may often be ex-

tracted by simple methods. Pearmain & Moor recommend that 50 grams be chopped fine and tied up in a muslin bag, which is placed in a water-bath. When the water is heated, the fat will generally run out clear. If not clear, it can be filtered through paper.

Henzold suggests the following: 300 grams of the powdered cheese are agitated in a wide-neck flask with 700 c.c. of 5% solution of potassium hydroxid previously warmed to 20°. In about ten minutes the cheese dissolves, the fat floats, and by cautious shaking may be collected in lumps. The liquid is diluted, the fat removed, washed in very cold water, keaded as dry as possible, melted, and filtered. It is claimed that the fat is not altered in composition by the process.

The fat of cheese may be estimated by the centrifugal method, as follows:

About 3 grams of the mixed cheese in small fragments are weighed and transferred to the bottle, the last portions being washed in with the acid of water. A few drops of ammonium hydroxid are added, and sufficient water to make the liquid about 15 c.c. The liquid is warmed with occasional shaking until the cheese is well disintegrated, and then treated as a sample of milk. The percentage of fat is found by multiplying the percentage reading by 15.45 and

dividing by the number of grams of cheese taken for analysis.

Chattaway, Pearmain & Moor use the following modification: 2 grams of the cheese are placed in a small dish and heated on the water-bath with 30 c.c. of concentrated hydrochloric acid until a dark, purplish-colored solution is produced. The mixture is now poured into the test bottle, portions of solution remaining in the dish rinsed with the hydrochloric acid fusel-oil mixture into the bottle, and, finally, enough strong hot acid added to fill the bottle up to the mark. It is then whirled for about a minute. The difficulty in this method is to get all the fat into the bottle. It is best to weigh the cheese in the bottle.

For accurate determination of fat, Revis and Bolton recommend the Schmid-Bondyzynski method, as follows: About 1.5 grams are weighed in a small flask, 5 c.c. of hydrochloric acid and a little powdered sulfur added and the mixture boiled gently. (For dry cheese, acid of sp. gr. 1.125 is best, for moist cheese, sp. gr. 1.19.) The mixture is cooled, transferred to the apparatus used for Röse-Gottlieb method, by the use of two portions of 2.5 c.c. each of alcohol and then small quantities of ether until 12.5 c.c. have been used. The contents are mixed, 12.5 c.c. light

petroleum added and the analysis carried out as described on page 72.

Lactose.—This may be estimated by boiling the finely divided cheese with water, filtering, and determining the reducing power of the filtrate on Fehling's solution.

Determination of Albuminoid Nitrogen (Stutzer's Method).—0.7 to 0.8 gram of the cheese are placed in a beaker, heated to boiling, 2 or 3 c.c. of saturated alum solution added to decompose alkaline phosphate, then copper hydroxid mixture (see below) containing about 0.5 gram of the hydroxid, and stirred in thoroughly; when cold, the mass is filtered, washed with cold water, and, without removing the precipitate from the filter, the nitrogen determined by the Kjeldahl-Gunning method. Before distillation, sufficient potassium sulfid solution must be added to precipitate the copper.

The special reagent is prepared as follows: 100 grams of copper sulfate are dissolved in 5000 c.c. of water, 25 c.c. of glycerol added, and then a dilute solution of sodium hydroxid until the liquid is alkaline. The mass is filtered, the precipitate is mixed well with water containing 5 c.c. of glycerol per liter, and washed until the washings are no longer alkaline. It is then rubbed up with a mixture of 90% water and 10% glycerol in sufficient quantity to obtain a uniform

magma that can be measured with a pipet. The quantity of copper hydroxid per c.c. should be determined. It should be kept in a well-closed bottle.

Ammonium compounds.—About 5 grams of cheese are rubbed up in a mortar with water, transferred to a filter, and washed with a liter of cold water. The filtrate is concentrated by boiling (if alkaline, it must be neutralized before heating), barium carbonate added, the liquid distilled, and the ammonium hydroxid in the distillate estimated by titration with standard acid.

According to Stutzer, magnesium oxid or magnesium carbonate (the latter usually contains some oxid) should not be used as some of the amino-compounds may be decomposed.

Amino-compounds.—The nitrogen as amino-compounds is estimated by subtracting from the figure for total nitrogen the sum of the protein and ammoniacal nitrogen. If nitrates are present, the nitrogen as such should also be determined and subtracted.

Van Ketel & Antusch propose the following methods for estimating the nitrogen compounds:

Ammonium compounds.—The sample, powdered with the addition of sand, is distilled with water and barium carbonate, and the distillate received in a measured quantity of standard

sulfuric acid, and, after boiling, the excess of acid is neutralized with standard sodium hydroxid, using rosolic acid as indicator.

Amino-compounds.—These are determined by macerating the powdered cheese in water for fifteen hours at the ordinary temperature. After adding a little dilute sulfuric acid (1 : 4), the proteins and peptones are precipitated by phosphotungstic acid. The precipitate is filtered off and washed with water containing a little sulfuric acid. The filtrate is made up to a definite bulk, and the nitrogen is determined in an aliquot portion of the liquid by the Kjeldahl-Gunning process, allowance being made for the nitrogen existing as ammonium.

Peptones and Albumoses.—These are determined jointly by boiling the powdered cheese (mixed with sand as before) with water and filtering from the undissolved casein and albumin. In an aliquot portion of the filtrate the peptones and albumoses are precipitated by adding dilute sulfuric acid and phosphotungstic acid. After washing with acidulated water the nitrogen in the precipitate is determined by the Kjeldahl-Gunning process.

The total nitrogen of the cheese is also determined, and after allowing for the nitrogen existing as other forms, the remainder is calculated to casein.

Poisonous Metals.—Lead chromate has been found in the rind of cheese, and finely divided lead in a number of Canadian cheeses. In England zinc sulfate has been employed under the name of cheese spice to prevent the heading and cracking. Arsenic has also been found; it may be detected by Reinsch's test. Lead, zinc, and chromium may be detected by ashing a portion of the sample in a porcelain crucible and applying the usual tests.

FERMENTED MILK PRODUCTS

The usual fermentation of milk is the conversion of the lactose into lactic acid, but by special methods other changes may be substituted. These modified fermentations are of rather ancient origin, and being produced by mixture of organisms, the products are complex and irregular. The proteins are more or less changed into proteoses and peptones.

Kumiss is milk which has undergone alcoholic fermentation. The inhabitants of the steppes of Russia prepare it from mares' milk. When cows' milk is used, sucrose must be added. It is often made by adding sucrose and yeast to skim-milk.

Vieth gives the following analysis of kumiss at successive stages of fermentation:

KUMISS FROM COWS' MILK

	ONE DAY	ONE WEEK	ONE MONTH	THREE MONTHS
Alcohol.....	1.1	0.9	1.0	1.1
Solids.....	11.3	8.9	8.6	8.5
Fat.....	1.6	1.4	1.5	1.5
Casein.....	2.0	2.0	1.9	1.7
Albumin.....	0.3	0.2	0.2	0.1
Carbohydrates.....	6.1	3.1	2.2	1.7
Lactic acid.....	0.2	0.9	1.3	1.9
Lactoprotein and peptone.	0.3	0.5	0.7	0.9
Soluble ash.....	0.1	0.2	0.2	0.2
Insoluble ash.....	0.4	0.3	0.3	0.3

The item "lactoprotein and peptone" refers to the substance precipitated by tannin after removal of the casein and albumin.

KUMISS FROM MARES' MILK

AT THE END OF:	ALCO- HOL	FAT	NITROGENOUS MATTERS	LACTIC ACID	LAC- TOSE	ASH
1 day.....	2.47	1.08	2.25	0.64	2.21	0.36
8 days.....	2.70	1.13	2.00	1.16	0.69	0.37
22 days.....	2.84	1.27	1.97	1.26	0.51	0.36

Kefyr.—This is usually made from cows' milk. It has been used in the Caucasus for centuries. For its preparation a peculiar ferment is used, which is contained in the kefir grains. These are first soaked in water, by which they are caused to swell and rendered more active, and then added to the milk. If taken out of the milk and dried, the grains may be used repeatedly.

The following are analyses of kefir:

KÖNIG HAMMARSTEN

Alcohol.....	0.75	0.72
Fat.....	1.44	3.08
Casein.....	2.88	2.94
Albumin.....	0.36	0.18
Hemialbumose.....	0.26	0.07
Peptone.....	0.04	
Lactose.....	2.41	2.68
Lactic Acid.....	1.02	0.73
Ash.....	0.68	0.71

According to König, good kefir will not contain more than 1% of lactic acid.

Analytic Methods.—Solids and ash are deter-

mined by evaporation as described on page 13. Acidity is determined by titration with $N/10$ alkali, using phenolphthalein or methyl-orange as an indicator. The amount of acidity is expressed in terms of lactic acid. The Kjeldahl-Gunning method will give the total nitrogen. For further examination of the nitrogenous bodies, the methods given on pages 106 to 108 may be applied. Total reducing carbohydrates may be estimated as given on page 32. If sucrose and common yeast have been added, the fermented material will be likely to contain invert-sugar, with unchanged lactose and sucrose, and the method of examination of sweetened condensed milk may be applicable. Fat can, probably in all cases, be determined with sufficient accuracy by the L-B. process. If it be desired to make polarimetric readings, the liquid should be clarified with acid mercuric nitrate solution (page 37), as some partly hydrolyzed proteins which have rotatory power may not be precipitated by other reagents. The determination of alcohol accurately is difficult, as the quantity is usually small. The cautious distillation of a considerable volume of the material previously neutralized with a little sodium hydroxid will yield a distillate in which alcohol may be detected and determined by the usual methods.

Preservatives are not likely to be used, since they would interfere with the fermentation, but attempts may be made to secure better keeping by adding some preservative after the fermentation has occurred. In some cases, therefore, tests for boric acid, formaldehyde, and salicylic acid should be made, as these will be most likely to be used.

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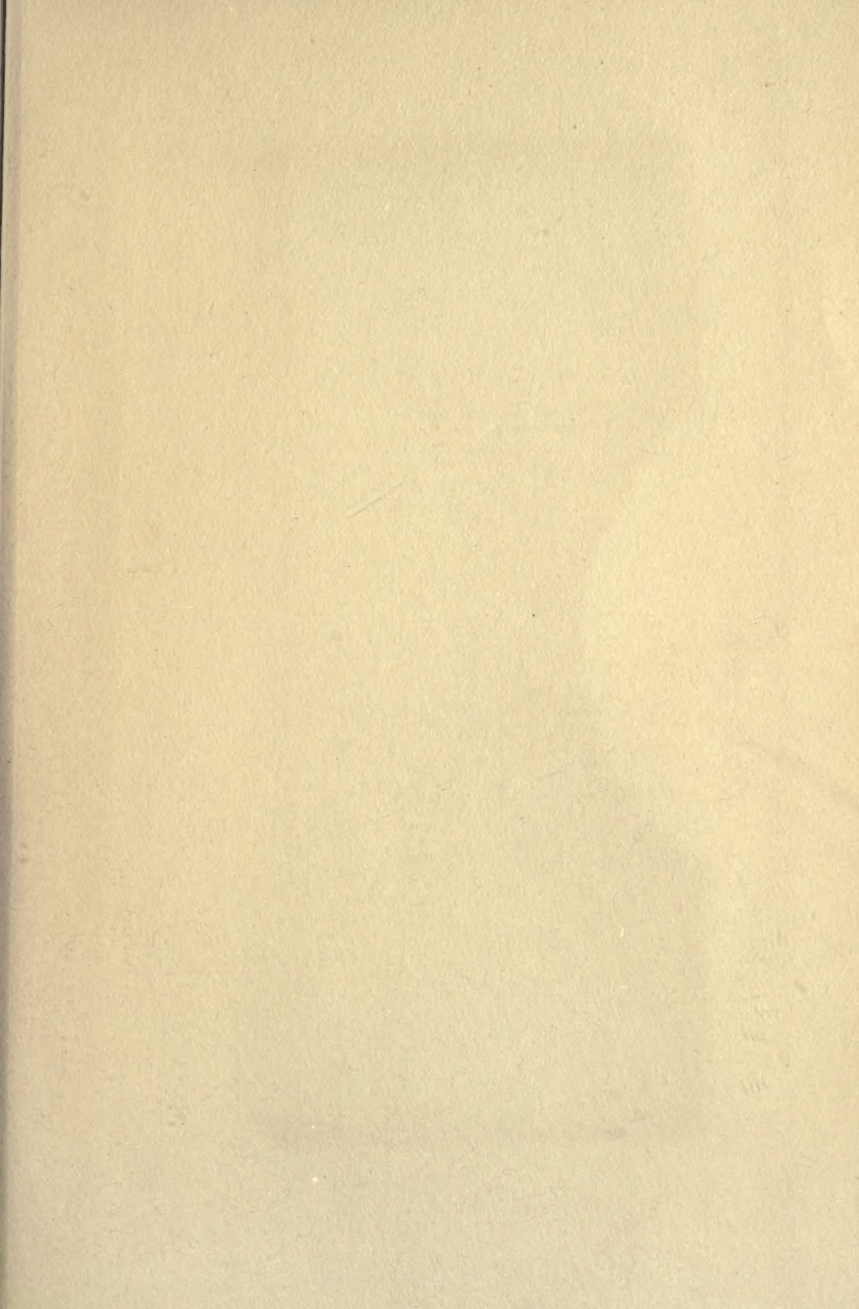
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